Interfering with neighbouring communities: Allelopathy astray in the tundra delays seedling development

Anna Katharina Pilsbacher¹ ², Bente Lindgård¹, Rigmor Reiersen¹, Victoria T. González³, Kari Anne Bråthen*¹

¹Department of Arctic and Marine Biology, UiT- Arctic University of Norway, N-9037 Tromsø, Norway
²Department of Language and Culture, UiT- Arctic University of Norway, N-9037 Tromsø, Norway
³Department of Ecosystems in the Barents region, Norwegian institute of Bioeconomy Research-NIBIO, Svanvik, Norway

*Corresponding author, telephone: +47 450 28754, e-mail: kari.brathen@uit.no

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Authors’ contributions
AKP and KAB conceived the ideas and designed methodology; AKP, KAB, BL and RR collected the data; AKP, KAB and BL analysed the data; KAB led the writing of the manuscript. All authors contributed critically to the drafts and gave final approval for publication.

Data availability
Data is accessible at UiT Open Research Data (https://opendata.uit.no, https://doi.org/10.18710/PGDJFB).

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Abstract

1. Altered species composition caused by environmental and climatic change can affect the transfer of plant residues among communities. Whereas transferred residues are typically considered a resource in recipient systems, residues of allelopathic species may instead cause interference.

2. Evergreen dwarf shrubs, specifically the allelopathic species *Empetrum nigrum* are increasing in abundance in response to a warming climate. *Empetrum* has small, evergreen leaves that can be transferred to other communities when withered and lost from the plant.

3. We hypothesize that *Empetrum* can have allelopathic effects in the recipient communities of the withered leaves. We call this allochthonous allelopathy as opposed to autochthonous allelopathy, which is well documented in communities where it grows.

4. We measured influx of allochthonous *Empetrum* leaves onto snow-covered snowbeds, where they are easily identified within the debris. Next, we compared the bioactivity of allochthonous withered leaves with that of green *Empetrum* leaves. Finally, we conducted an experiment testing the germination and seedling growth of ten tundra species in snowbed soil supplemented with no (control) and three densities of allochthonous *Empetrum* leaves.

5. We found *Empetrum* leaves to be common on the snow cover of snowbeds. We found *Empetrum* leaves collected on snowbeds to be as bioactive as green leaves. Finally, we found forb species to have reduced germination and all ten species to have delayed seedling development when growing in snowbed soil supplemented with withered *Empetrum* leaves. Seedlings under the control treatment were 2.3 times longer and had 3.2 times more leaves in comparison to seedlings grown under the strongest allochthonous leaf treatment.

6. Results from our study imply that *Empetrum* is allelopathic in recipient systems of its allochthonous leaves. The abundant nature of *Empetrum* in the tundra, suggests that allochthonous allelopathy is a common phenomenon, causing biotic stress in snowbeds and potentially other parts of the tundra. Exemplifying the ability of a plant to interfere in neighbouring communities, our study demonstrates a plant trait that may provide insight to other study systems.
Introduction

Movement of plant detritus is ubiquitous and can strongly influence ecosystem dynamics in neighbouring systems (Polis, Anderson & Holt 1997). Altered species composition caused by environmental and climatic change can therefore have consequences beyond their particular systems. The movement of terrestrial detritus into riparian systems, typically termed allochthonous input, can represent important nutrient resources subsidizing both freshwater food webs and neighbouring plant communities (Xiong & Nilsson 1997). Likewise, plant detritus boosts the nutrient and energy availability in terrestrial recipient systems, such as aeolian ecosystems where plant detritus is an essential resource (Polis, Anderson & Holt 1997). However, there is considerable variation in both the quality of plant residues (Cornwell et al. 2008), and in the fate of plant residues in any given recipient system (Freschet, Aerts & Cornelissen 2012). The effect of allochthonous plant detritus, or “away-field” detritus (sensu Ayres et al. 2009), is therefore highly context dependent. Identifying the quality and quantity of plant residues moving between systems can provide a better understanding of ecosystem dynamics and modification under environmental and climatic change.

The capacity to be a source of allochthonous plant detritus varies between species causing recipient systems to receive a selection of the leaf litter produced in source systems. Species whose withered parts possess traits facilitating the movement with physical forces such as wind and water (Polis, Anderson & Holt 1997) are likely to be more common in the plant detritus. The source species and plant parts of allochthonous detritus are therefore not random, but rather dominated by plants with adapted traits.

After deposition, the traits contained in the detritus can affect the recipient system. Allochthonous as opposed to autochthonous litter may have a lower content of nutrients and secondary metabolites depending on the extent to which the litter is disintegrated during movement. In addition, decomposition rates, and hence the release of nutrients and secondary metabolites, can be lower in the recipient system due to the soil biota not being adapted to the litter (Veen et al. 2015). This suggests allochthonous plant detritus is in general of low nutrient quality in recipient systems. However, secondary metabolites can still be active in leaves after they have withered (Hättenschwiler, Tiunov & Scheu 2005). Secondary metabolites in allochthonous plant litter may thus modify the environment of the recipient system through processes of ecosystem engineering or niche construction (Odling-
Smee et al. 2013). Depending on whether the resulting niche construction is of benefit to the source species, by creating a suitable, future habitat, allochthonous detritus with secondary metabolites may even be selected for (Matthews et al. 2014). In summary, plant species that produce allochthonous plant detritus may affect communities in which they do not grow mostly through their secondary metabolites. We term this allochthonous niche construction.

Here we address *Empetrum nigrum* as a candidate for allochthonous niche construction by means of environmental modification through allelopathy (Figure 1). *Empetrum* is a dwarf shrub common in heaths of circumboreal-polar areas (http://nhm2.uio.no/paf/) and is allelopathic in its home-soil (Nilsson & Zackrisson 1992; Bråthen, Fodstad & Gallet 2010). The allelopathic capacity of *Empetrum* leaves is attributed to the production of secondary metabolites, most notably the dihydrostilbene Batatasin-III (Odén et al. 1992; Wallstedt et al. 1997; Nilsson, Gallet & Wallstedt 1998) in the glands of its small, evergreen leaves (Muravnik & Shavarda 2012, Figure S1 in Supporting Information). Batatasin-III is temporarily stable, resistant to microbial degradation (Wallstedt et al. 1997; Wallstedt, Gallet & Nilsson 2005) and resists change in response to altered environmental conditions (González et al. 2015). The withered leaves of *Empetrum* decompose slowly (Tybirk et al. 2000; Parker et al. 2018) and retain allelopathic secondary metabolites, although at lower concentrations than in green leaves (Gallet, Nilsson & Zackrisson 1999). Copious amounts of *Empetrum* litter can accumulate as reported from a boreal forest site where the average litter abundance was 425 ±60 g/m² (Wallstedt et al. 2000). With an average leaf weight of 0.53 mg (± 0.11 mg) (Appendix 1 in Supplementary Information) the leaves are comparable to intermediate sized tundra seeds (González et al. 2010). It thus can be inferred that the leaves are able to disperse with both fluvial and aeolian processes (Figure 1) and accumulate on snowbeds like seeds (Larsson & Molau 2001) (Figure S2). In summary, *Empetrum* might have capacity for allochthonous niche construction when its leaves are transported to other communities (Figure 1).

The tundra is changing in response to a warming climate, particularly through shrub encroachment (Olofsson et al. 2009; Bråthen et al. 2017; Vowles & Björk 2019). Studies on ongoing and predicted changes often focus on responses to abiotic (warming, changing snow conditions, nutrients) and biotic factors (herbivory) (Björkman et al. 2020). However, in the tundra, species composition and richness can be more strongly linked to dominant plant species than to changes in abiotic factors and herbivory (le Roux et al. 2013). Furthermore, these dominant
species are often favoured by climate warming (Wookey et al. 2009). Empetrum is one of these dominant species, responds positively to warming, and its presence is a predictor of species presence and/or species richness in the tundra (Pellissier et al. 2010; Ravolainen et al. 2010; le Roux et al. 2014; Bråthen, González & Yoccoz 2018). By addressing allochthonous niche construction, this study explores the potential for Empetrum to also be a predictor in neighbouring communities. We focus on snowbeds, because they are affected by climate change (Björk & Molau 2007). With an ongoing decline in the duration of snow cover by 30% (Box et al. 2019), changes in vegetation composition are already considerable (Wipf & Rixen 2010). Here we ask whether snowbed communities can be affected by Empetrum through allochthonous allelopathy.

We performed our study in three parts. We quantified allochthonous Empetrum leaves on snowbeds, we assessed bioactivity in allochthonous Empetrum leaves collected on the snow-cover of snowbeds and compared it to the bioactivity of green leaves. Finally, we conducted an experiment testing germination and seedling growth of ten tundra species in snowbed soil treated with allochthonous Empetrum leaves. We predicted that; I) Allochthonous Empetrum leaves exist in snowbed environments. II) The bioactivity of allochthonous Empetrum leaves is similar to that of green leaves. III) Germination and plant growth of tundra species will decrease with increasing concentration of allochthonous Empetrum leaves added to their soil, independent of their growth form and habitat affinity.
Figure 1. A conceptual presentation of the allelopathic effect of Empetrum in recipient snowbed communities.

Soil beneath Empetrum is allelopathic (Nilsson & Zackrisson 1992; Bråthen, Fodstad & Gallet 2010). The secondary metabolites in Empetrum leaves are released as leachates from both fresh senescing and withered leaves (brown leaves, autochthonous litter) during snowmelt and rain events (Brännäs et al. 2004) (shaded area). The withered leaves have not lost their allelopathic capacity (Gallet, Nilsson & Zackrisson 1999). If the withered leaves are transported to neighbouring habitats through wind, snowdrift, or meltwater streams (Körner 2003), we hypothesize they can cause allochthonous allelopathy in recipient systems.
Material and Methods

Study sites

The study sites were located across Northern Fennoscandia, with particular sampling efforts above the treeline at the mountain pass of Ifjord (Ifjordfjellet), Finnmark county (250–450 m asl) for the snowbed study, and in tundra sites in northern Troms county (50–600 m asl) for the sampling of soil, leaves and seeds (Figure 2). Bioactivity tests, chemical analyses and the phytotron experiment were conducted at UiT - The Arctic University of Norway.

Figure 2. Map of sampling locations. Locations of the snow bed study were at Ifjordfjellet (purple dots in inset). Sampling locations of leaves for the bioactivity assessment were distributed over several sites across Northern Fennoscandia (bluegreen dots). Sampling locations for seeds, soil and dead leaves for the phytotron experiment, were situated in Troms (light green dots). Leaves for the bioactivity assessment were also sampled at Ifjordfjellet (no specific locations marked).
**Snowbed study**

The study of allochthonous *Empetrum* leaves on snowbeds was conducted within an area of 20 km² of Ifjordjellet during five days in late June 2013 (Figure 2). Typical habitats of this tundra area are dwarf shrub heaths dominated by *Empetrum nigrum*, *Vaccinium myrtillus* and *Betula nana* and snowbeds dominated by acrocarp bryophytes such as *Dicranum* spp., vascular plants such as the prostrate shrub *Salix herbacea*, small forbs such as *Sibbaldia procumbens* and *Omalotheca supina* and graminoids such as *Anthoxanthum nipponicum*.

All accessible snowbeds in the area were included in the study as long as they had a snow cover larger than 20 m² (size set to avoid sampling only small snowbeds). Detritus accumulates in non-uniform patterns on snowbeds (Figure S2). Therefore, we chose a subjective placement of 40 cm × 40 cm plots within each snowbed on the patches with the highest detritus concentration. In each plot, the presence and abundance of *Empetrum* leaves in the accumulated detritus was recorded as one of five categories (0, 1-10, 10-20, 20-30, and more than 30 leaves). On average 8 plots per snowbed (with a minimum of four and a maximum of ten plots) were examined for a total of 408 plots on 45 snow-covered snowbeds.

To assess whether the influx of leaves was dependent on the distance to the nearest *Empetrum* source, we measured the distance between the snowbed’s edge to the closest area of *Empetrum* dominance. The distances measured were exact to the meter up to 20 meters, after which distances were measured to the closest 10 m.

For an assessment of the importance of snowbed slope for the accumulation of leaves, the snowbeds’ slope was measured in the centre of their snow cover.
Bioactivity and chemical content of allochthonous leaves

We conducted bioassays on Empetrum leaves collected from two snow-covered snowbeds at Ifjordfjellet in 2009 to ascertain whether allochthonous Empetrum leaves retain bioactivity. In addition, we analysed their Batatasin-III and total phenolics content. For a comparison of allochthonous leaves and green leaves, we ran bioassays and chemical analyses of green Empetrum leaves collected at Ifjordfjellet (n=8) (Figure 2). Finally, to assess whether our findings from Ifjordfjellet are representative for the larger region, we collected and tested green leaves across Northern Fennoscandia (n=12).

The bioassays and the Batatasin-III analysis were conducted using methodology described in González et al. (2015). For each collection site, approx. 25 mg of leaves were placed in each of two Ø 4.5 cm Petri dishes under two pieces of Whatman No 1 filter paper. The filter papers were moistened with 1.5 ml distilled water and 10 germinated lettuce (Lactuca sativa) seedlings were transferred onto them. Then the lid was attached with parafilm. Three Petri dishes without Empetrum leaves served as controls. After five days, average root length per Petri dish was calculated.

Batatasin-III was extracted from 25 mg of dried leaves with ethyl acetate. Extracts were evaporated to dryness with Speedvac concentrator and Rotary evaporator. Dried residue was dissolved in 1 ml acetonitril:water 1:1 (V:V), acidified with 0.5% acetic acid and filtered with a 0.45 µm Millipore filter. Separation and quantification of Batatasin-III was performed with gradient high-performance liquid chromatography (HPLC) and DAD detection using a 996 Photodiode array (PDA) detector. Batatasin-III was separated on Waters x-bridge C18-5 µm particle size column (4.6 x 150 mm) (part no. 186003116) at 30°C, with injection volume of 20 µl, flow rate of 1ml min⁻¹, and retention time of 20.8 min. Batatasin-III was confirmed using an internal standard and quantified using an appropriate calibration curve produced by the Waters Alliance chromatographic system. The wavelength measured was 273.2 nm. Batatasin-III was determined using a gradient of mobile phases with milliQ water and acetonitrile, both acidified with 0.5% acetic acid.

The phenolic analysis was based on the same extract as the Batatasin-III analysis. We measured total phenolics by the Folin-Ciocalteau colorimetry method. We followed the basic protocol 1 in Current Protocols in Food.
Analytical Chemistry (Waterhouse 2002), with Gallic acid as standard and reading absorbance at 750 nm using a Spectra MAX 250.

**Abundance of Empetrum**

Using published survey data (Bråthen & Lortie 2016a), we compared the abundance of *Empetrum* at Ifjordfjellet, to that of other regions in Fennoscandia to assess whether the density of allochthonous leaves found in the snowbed study could be representative to that of other tundra regions. In the survey, *Empetrum* abundance was measured as point intercepts and converted to biomass. Because these measures of *Empetrum* abundance are a minor part of this particular study, we refer to previously published papers for an explanation of the study design (e.g. Bråthen & Lortie 2016b).

**Collection of Empetrum leaves, seeds and soil for phytotron experiment**

*Empetrum* leaves, soil and seeds for the phytotron experiment were sampled at various sites in Troms county, Norway, in between July and early October 2012 (Figure 2). Withered leaf samples were collected in areas where *Empetrum* was a dominant species. Soil and seeds were collected from sites with 500 m minimum distance to *Empetrum* dominated areas.

Because of the focus on dispersed *Empetrum* leaf litter, all leaves for the phytotron experiment were collected from branches already detached from the mother plant or branches showing clear signs of withering. Leaf samples were stored dry and at room temperature. A pre-experiment assessment of the withered leaves demonstrated that they were clearly bioactive (Appendix 2).

Plant species representing different growth forms (forbs, woody plants or graminoids) and habitat affinities (snowbed or more generalist), had to be present in at least three sites to justify seed collection. Consequently, the seeds of the forbs *Bistorta vivipara, Omalotheca supina, Pedicularis lapponica, Sibbaldia procumbens* and *Solidago virgaurea*, the grasses *Anthoxantum nipponicum, Avenella flexuosa* and *Nardus stricta*, and the woody species *Chamaepericlymenum suecicum, Dryas octopetala, Empetrum nigrum, Salix herbacea* and *Vaccinium myrtillus* were gathered. We refer to Pan Arctic Flora database ([http://nhm2.uio.no/paf/](http://nhm2.uio.no/paf/)) for nomenclature and Lid and Lid (2005) for categorization of species according to habitat. We collected approximately 50 seeds or 25 inflorescences for each species present at a given site. The range of seed collection sites ensured that seeds
represent different populations and environmental contexts. Seeds were stored at room temperature until cleaned and thereafter frozen at -10°C.

We tested the baseline germination rate using 100 seeds per species (Appendix 3). The low germination rates of *Pedicularis, Chamaepericlymenum, and Empetrum* excluded them from further experiments.

Soil was collected from snowbeds in the vicinity of five seed collection sites (Figure 2). This geographic range ensured variation in environmental contexts. Preference was given to snowbeds with deeper organic layers and sparse vegetation covers. This ensured collecting a sufficient amount of soil organic matter and avoided soil with high root density. First, a soil core (Ø 5 cm, 10 cm deep) was taken. If the soil profile showed at least 6 cm of non-sandy topsoil, 200-250 grams of topsoil was collected at each of 10 subsites and the litter and moss layer discarded. The collected soil was dried at room temperature and sieved with a 4 mm sieve to remove non-organic matter and roots. Soil from all subsites within a site was pooled and stored at -10°C. Soil analysis (Appendix 4), showed that the sites were comparable in soil quality (Table 1).
Table 1. Description of the snowbed soils included in the greenhouse experiment in terms of soil pH and soil element concentration (average ± SD of soils from five collection sites).

<table>
<thead>
<tr>
<th>pH</th>
<th>P (%)</th>
<th>K (%)</th>
<th>N (%)</th>
<th>C- org (%)</th>
<th>C-inorg (%)</th>
<th>Water (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>4.52 ± 0.17</td>
<td>0.0013 ± 0.0001</td>
<td>0.0102 ± 0.0013</td>
<td>0.96 ± 0.08</td>
<td>43.6 ± 3.3</td>
<td>13.6 ± 1.6</td>
<td>42.8 ± 1.7</td>
</tr>
</tbody>
</table>

Estimation of abundance of Empetrum leaves in soil from snowbeds

We measured the abundance of Empetrum leaves in two soil cores (Ø 5.5 cm, 10 cm deep) at the snow cover edge of each of the five snowbeds sampled for soil, to ascertain the concentrations of Empetrum leaves in snowbed soils.

The soil cores were weighed, dried at 95°C for 12 hours and sieved. All Empetrum leaves found during sieving were counted. Importantly the leaves occurred throughout the soil core, indicating that leaves become incorporated over time. Using the approximation of 1 leaf = 1 mg, leaf abundance within 1 kg of soil was calculated. Leaf abundance in soil varied from 10 g to 43 g kg⁻¹ soil, with 6 out of 10 samples having 25 - 35 g kg⁻¹ soil. From this, we established a treatment gradient with four levels at 0, 15, 30 and 45 g Empetrum leaves kg⁻¹ soil at field capacity.

Phytotron experiment

The experiment was conducted in autumn 2012 in the phytotron at UiT - The Arctic University of Norway.

First, the frozen soil was thawed in a dark room at 4°C for three days and mixed with sand at a 1:5 ratio as suggested by Medina-Roldán, Paz-Ferreiro and Bardgett (2012) to remove site-specific differences. The soil was placed in a dark room for 48 hours at 0.5°C and then quartered. One part was kept as control (0 g leaves kg⁻¹ soil), while the remaining parts were mixed with dry, withered Empetrum leaves in the previously established treatment concentrations. The soil was then transferred into standard cylindrical planting pots (Ø 9 cm, 7.5 cm deep) for a total of 20 pots per treatment.
Seeds were thawed and planted for a total of ten seeds in each of two pots per species and treatment. The soil was covered with fine grade Perlite© to avoid water loss. In order to break seed dormancy, the seeds were stratified in a darkened room at 0.5°C for 7 days. Seeds were left to germinate under 24 hours artificial light at 8°C (simulating the low-alpine light and temperature of Northern Fennoscandia).

The seeds were visited every three days for watering and germination assessment. After the first seed germinated in a pot, seed germination was registered for a period of 25 days and summed for a maximum of 10 per pot.

All germinated seedlings above the first five were removed from pots to avoid seedling competition. The remaining five seedlings were allowed to grow for 25 days after which their height and number of secondary leaves was recorded. The pot-specific germination of the ≥5 first seedlings was synchronized within the tree day interval between visits. Thus, no measures to control for varying seedling ages within pots was conducted.

**Data Analysis**

We analysed the data using linear mixed effects models (Pinheiro & Bates 2000) in the R environment version 3.4.4 (http://www.r-project.org). We used the lme package for statistical analysis and the ggplot2 package for the graphic representation of data. Whenever data did not conform to normal distribution, they were transformed for the analysis and back-transformed for the presentation of model predictions.

The average density distribution of *Empetrum* leaves within snowbeds was displayed according to the five leaf density categories. The density of *Empetrum* leaves on snowbeds in response to distance from *Empetrum* heath and snowbed slope was modelled with the leaf density as a fixed factor and the identity of snowbeds as random factor. The leaf density categories were treated as a continuous variable from 0 to 4. The interaction between distance and slope did not improve the model and was excluded from the final model.

The test of bioactivity and chemical content dependence on leaf source (allochthonous or green *Empetrum* leaves), used leaf source as fixed factor and sampling locations as random factor. Testing bioactivity and chemical content dependence on region (Ifjord vs other sampling regions within Northern Fennoscandia), we used region (Ifjord vs other Fennoscandian) as a fixed factor and the location of all regions as random factor.
For the analysis of *Empetrum* abundance in Ifjord vs other regions within Northern Fennoscandia, we used region (Ifjord vs other Fennoscandian) as fixed factor and the hierarchical sampling levels of the study design in Bråthen and Lortie (2016b) as random factors.

Both the germination test and the seedling growth test were modelled with treatment as a continuous predictor. When testing whether the treatment effect was dependent on growth form (forbs, grasses and woody) and/or habitat affinity (heath, snowbed, general), we used treatment in interaction with growth form and habitat affinity as fixed factors. For each model Pot ID nested in species identity was applied as random factor. Growth form or habitat affinity were not retained in models where they did not explain variation in treatment effects.

To display the effect of treatment on the species level, we used the ggplot2 package with linear model fit.

**Results**

**Snowbed study**

*Empetrum* leaves were found on the snow cover of all 45 surveyed snowbeds, in the majority of plots per snowbed and, for most plots, in medium to high abundance (Figure 3). Leaf density on snowbeds was independent of snowbed slope (effect size - 0.009 ± 0.016 CI) and distance from *Empetrum* heath (effect size - 0.007 ± 0.008 CI) (Figure 3).
Figure 3. Density of *Empetrum* leaves on the snow cover of 45 snowbeds at Ifjordfjellet (A). Boxplots display outliers more than 1.5 times the interquartile range. Average density of *Empetrum* leaves on the snow-covered snowbeds in response to distance from the snowbed edge to the nearest *Empetrum*-dominated vegetation (B).

**Bioactivity and chemical content of allochthonous leaves**

We found similar bioactivity in green and allochthonous leaves in terms of how they affected lettuce root length (effect size 0.128 ± 0.430 C.I.), and that the lettuce roots in the controls (no leaves) was considerably longer than that of lettuce affected by the allochthonous leaves (effect size 1.874 ± 0.700 C.I.) (Figure 4). However, the leaf content of phenols was clearly highest in green leaves (effect size 11.142 ± 3.716 C.I.) and although not significant, the leaf content of Batatasin-III was also higher (effect size 7.536 ± 13.643 C.I.) (Figure 4).
Figure 4. Comparison of the bioactivity and chemical content of Empetrum leaves of allochthonous (on snowbeds) and autochthonous (green leaves on plants) source. The bioactivity assay (A) includes a control treatment (no leaves). Chemical content of Batatasin-III (B) and total Phenols (C) in allochthonous and green leaves. Boxplots display outliers more than 1.5 times the interquartile range.

Comparison of green leaves between Ifjord and other regions in Fennoscandia

In order to evaluate whether results from Ifjordfjellet (the site of the snowbed study and of the collection of leaves for comparison between green and allochthonous leaves) were representative, we compared Empetrum abundance, bioactivity and chemical content of green, fresh leaves in Ifjord to that of other regions in Northern Fennoscandia.

Empetrum was present in 87% of plant communities in Ifjord and 85% of plant communities in other regions, suggesting that Empetrum is extraordinarily common in the low alpine areas of Ifjord and other regions of Fennoscandia (Bråthen et al 2017). The abundance of Empetrum in terms of biomass was similar between the regions (effect size 2.771 ± 10.164 C.I.) (Figure S3).

Green leaves from Ifjord were more effective in limiting lettuce root length than green leaves from other regions in Northern Fennoscandia (effect size 3.911 ± 1.667 C.I.) (Figure S3). The leaf content of Batatasin-III, however,
was comparatively lower in *Empetrum* leaves from Ifjord (effect size - 13.771 ± 6.664 C.I.). There also was a
tendency for lower phenol content at Ifjord (effect size - 16.795 ± 24.575 C.I.) (Figure S3). Thus, results of
bioactivity and chemical content were inconsistent in indicating the direction of difference in the allelopathic effect
by *Empetrum* leaves from Ifjord in comparison to other regions in Fennoscandia, indicating there are potentially
other compounds having effect that were not studied here. Overall the results indicate that *Empetrum* leaves are
bioactive and contain both Batatasin-III and phenols across Northern Fennoscandia.

**Greenhouse experiment**

Seed germination was reduced by the allochthonous leaf treatment, but only among forb species (Table 2, Figure
5). Among forbs, the treatment effect corresponded to the sum of germinated seeds being reduced from 7.9
seeds under control conditions, to 7, 6.2 and 5.3 germinated seeds under the 15 g, 30 g and 45 g allochthonous
leaf treatment respectively. This reduction corresponds to a 1.5-fold, or 33% decrease between the control and
the strongest allochthonous leaf treatment. There was no modification of treatment effect by habitat affinity (effect
size 0.026 ± 0.042 C.I.).
Figure 5. Sum of germinated seeds per pot per growth form in response to treatment with *Empetrum* leaves in soil.

Seedling growth was reduced by the allochthonous leaf treatments in terms of the number of secondary leaves and leaf height (Table 2, Figure 6). The seedlings had an average of 3.9, 2.5, 1.8 and 1.2 leaves and an average of 2.8, 1.9, 1.6 and 1.2 cm height under the control (0 g), 15 g, 30 g and 45 g allochthonous leaf treatment respectively. In total, seedlings under the control treatment were 2.3 times longer and had 3.2 times more leaves in comparison to seedlings grown in the strongest allochthonous leaf treatment.

Leaf number was clearly affected by treatment independent of growth form (effect size of grass 0.008 ± 0.029 C.I. and woody 0.029±0.032 C.I. vs that of forbs) or habitat affinity (effect size of snowbed -0.029 ± 0.029 C.I. vs generalist species). Similarly, growth expressed as leaf height was clearly affected by treatment independent of growth form (effect size of grass -0.012 ± 0.024 C.I. and woody 0.002±0.026 C.I. vs that of forbs) or habitat affinity (effect size of snowbed -0.004 ± 0.024 C.I. vs that of generalists).
Figure 6. Growth response of seedlings of a range of species to treatment with Empetrum leaves added to their soil. Growth responses are presented in terms of number of secondary leaves (A) and height (B).

Table 2. Effect size (±95% C.I.) of treatment effects of allochthonous leaves on the sum of germinated seeds and seedling growth. Growth was measured as the number of secondary leaves and leaf height. Estimates from mixed models with species and Pot ID as random factors.

<table>
<thead>
<tr>
<th>Response</th>
<th>Interacting factor</th>
<th>Effect size (± 95 % C.I.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>GERMINATION</td>
<td>Forbs</td>
<td>-0.056 (-0.084, -0.028)</td>
</tr>
<tr>
<td></td>
<td>Grasses</td>
<td>-0.002 (-0.035, 0.030)</td>
</tr>
<tr>
<td></td>
<td>Shrubs</td>
<td>-0.003 (-0.036, 0.029)</td>
</tr>
<tr>
<td>GROWTH</td>
<td>Number of leaves</td>
<td>-0.057 (-0.069, -0.044)</td>
</tr>
<tr>
<td></td>
<td>Height</td>
<td>-0.034 (-0.043, -0.024)</td>
</tr>
</tbody>
</table>
In this study we find evidence of allochthonous allelopathy using the model species *Empetrum nigrum*. First, a high concentration of *Empetrum* leaves was found in the debris on snow beds distanced 3 to 50 meters away from *Empetrum* heath, establishing *Empetrum* as a source of allochthonous detritus into snowbed environments. Second, allochthonous leaves were as bioactive as green leaves in reducing the growth of lettuce seedlings. Although at lower concentrations than green leaves, the allochthonous leaves contain secondary metabolites associated to the allelopathic capacity of *Empetrum* (Gallet, Nilsson & Zackrisson 1999, this study). Third, both seed germination and seedling growth of a range of tundra plant species negatively responded to allochthonous *Empetrum* leaves in soil at concentrations likely to occur in snowbed soils. Surprisingly, germination was negatively affected only in forb species. Our findings suggest that *Empetrum* can reduce primary productivity at vital life stages of vascular plant species in tundra snowbeds through allochthonous allelopathy.

Delaying the development of plant species in recipient systems of allochthonous *Empetrum* leaf litter, may be at *Empetrum*’s benefit and even selected for. If the recipient system represents a potential habitat, modifying the environment to the disadvantage of other species result in benefits to the niche constructor (Matthews *et al.* 2014). *Empetrum* establishment in snowbeds is likely. *Empetrum* berries are dispersed by zoochory of migratory animals such as reindeer (Bråthen *et al.* 2007a), providing opportunities for establishment in new habitats. In snowbeds, reduced competition for light and other resources by the extant flora in response to the allochthonous allelopathy, could facilitate the establishment of slow growing species such as *Empetrum*. Because it is dispersed by endozoochory, resources in the faeces could further boost *Empetrum* growth and reduce the allelopathic impact of the allochthonous *Empetrum* leaves present (Bråthen, Fodstad & Gallet 2010). The latter process also facilitates the establishment of other species dispersed with zoochory. In contrast, in areas unsuitable as new habitats, for instance late snowbeds with insufficient growth season length for *Empetrum*, the allochthonous allelopathy could contribute to the appearance of barren snowbeds with low productivity.

Batatasin-III “becomes physically trapped by organic matter” (Wallstedt, Gallet & Nilsson 2005) which neutralises its allelopathic effect (González *et al.* 2015). For this reason, snowbed soil rich in organic matter content might absorb and neutralize the allelopathic effect of Batatasin-III. However, if Batatasin-III is retained in low degradable
leaves, prevented from being trapped by the organic soil (Parker et al. 2018) and released in leachate pulses

whenever water drains the soil, it may take substantial organic material such as mammalian faeces or even fire, to neutralize its bioactivity (Bråthen, Fodstad & Gallet 2010). Wallstedt et al. (2000) found indications that

Batatasin-III can disturb membrane integrity and, consequently, disrupt fundamental processes such as ion uptake from roots. Perhaps for this reason, signs of reduced growth were observed for all species only after

seedlings had started growing and their roots came in contact with the Batatasin-III reservoir.

This study found delayed development at the seedling stage in common species from three growth forms when growing in snowbed soil with withered leaves of Empetrum. The finding corresponds a study on the effect of

Batatasin-III, where reduced germination and seedling growth for a range of tundra growth forms was observed (González et al. 2015). It is noteworthy that the deciduous shrub Betula nana and the evergreen dwarf shrub Vaccinium vitis-idea—species encroaching into snowbed environments in response to climate warming along with Empetrum (Vowles et al. 2017) — also experienced reduced germination and seedling growth in response to Batatasin-III (González et al. 2015). Empetrum might, therefore, delay seedling establishment of other shrub species and their encroachment through allochthonous allelopathy. It remains unknown, whether Empetrum gains enough competitive advantage through allochthonous allelopathy to outcompete other expanding shrubs or whether Empetrum simultaneously delays its own establishment, thereby negating any competitive advantage.

Snowbeds are among the habitats most affected by climate change (Björk & Molau 2007). The decline in the duration of snow cover is likely to cause a reduction in forbs that, in contrast to species of other growth forms, benefit from late snowmelt and are hence most vulnerable to earlier snowmelt (Wipf & Rixen 2010). In addition, results from this study indicate that forbs are most vulnerable to allochthonous allelopathy by Empetrum, exhibiting reduced germination rates in addition to reduced seedling growth. Yet, importantly, all growth forms were equally affected by allochthonous allelopathy by Empetrum at their seedling stage, which suggests overall reduced growth. Plants in snowbeds represent an important resource to many tundra herbivores (review in Björk & Molau 2007). Changes in vegetation composition and productivity will therefore also have consequences to trophic aboveground interactions. In particular, lemmings, depending on snowbeds for their winter-habitat, might be affected. Yet, lemming activity could also cause disturbances that promote seedling density richness in snowbeds (Nystuen et al. 2014) and counteract the allelopathic effects by Empetrum with their faeces. Such
disturbance may, in turn, benefit forbs, that are found to be the most disturbance resistant growth form (Evju, Hagen & Hofgaard 2012). In summary, snowbeds are affected by a multitude of factors. Here, we identify allochthonous allelopathy as an additional factor to be considered when predicting snowbed change.

Our results suggest that allochthonous allelopathy in snowbeds is a phenomenon to be expected over large spatial scales in Northern Fennoscandia and other regions where Empetrum is common. The extent of allochthonous allelopathy is likely to increase as Empetrum is advancing in response to climate change (Bråthen, González & Yoccoz 2018; Vowles & Björk 2019) and resistant to climatic variability (González et al. 2019). Furthermore, although Empetrum is vulnerable to trampling (Tybirk et al. 2000), it is not decreasing in response to higher animal densities (Bråthen et al. 2007b), and can even increase in the presence of mammalian herbivores (Vowles et al. 2017). Our study focused on snowbeds as recipient system. It is likely that Empetrum can be a source for allochthonous detritus to other habitat types, albeit with lower influx rate of leaves and a weaker allochthonous allelopathic effect. Large-scale, observational studies suggest that the contribution of autochthonous allelopathy by Empetrum is substantial (Ravolainen et al. 2010; Bråthen & Ravolainen 2015; Mod et al. 2016), lowering the species richness and abundance of herbaceous vascular plants (Bråthen, González & Yoccoz 2018). However, Empetrum also explains biodiversity patterns at spatial scales at the landscape level (Ravolainen et al. 2010), suggesting allochthonous allelopathy is also of considerable importance.

**Conclusion**

Changes in the tundra are ongoing. These changes are varied and not always as predicted (Björkman et al. 2020). This indicates there are several contributing factors and processes that still need to be established. We suggest that one such factor is allochthonous allelopathy, which, along with niche construction by dominant plants in their home communities, can represent a confounding factor to the consequences of environmental and climatic change. Allochthonous allelopathy can be a common and increasing phenomenon, as indicated by the abundance of Empetrum, causing biotic stress in snowbeds and other parts of the tundra. Furthermore, allochthonous allelopathy may modify the capacity of existing and novel species in the tundra to encroach further.
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650-655.


SUPPORTING INFORMATION

Additional supporting information may be found in the online version of this article.

Figure S1. Small glands on the Empetrum nigrum leaves and molecular formula of Batatasin III

Figure S2. Photocollage depicting the process of transport and accumulation of allochthonous Empetrum nigrum leaves

Figure S3 Comparison between Ifjord and other regions within Northern Fennoscandia

Figure S4. Root length of lettuce seedlings in response to exposure to different amounts of Empetrum nigrum leaves

Table S1. Soil content description for each of the five sites included in the greenhouse experiment.

Appendix 1. Leaf traits enabling dispersal

Appendix 2. Pre-experiment assessment of bioactivity of leaves

Appendix 3. Baseline germination rate

Appendix 4. Soil analysis
Figure S1. Small glands on the *Empetrum nigrum* leaves and molecular formula of Batatasin III. A photograph depicting the white-looking small glands on the *Empetrum nigrum* leaves where Batatasin-III can be found, and the formula of the dihydrostilbene Batatasin-III. Photograph taken by last author.
Figure S2. Photocollage depicting the process of transport and accumulation of allochthonous *Empetrum nigrum* leaves. A. *Empetrum* can cover vast areas and dominate in the vegetation. B. Autochthonous withered leaves of *Empetrum* can be attached to the plant but are easily detached when touched or when exposed to strong winds. C. Detritus on snowbed surfaces contain withered leaves of *Empetrum*. D. When the snow cover of snowbeds melts out, vast amounts of accumulated detritus may be exposed. E. The abundance of detritus can be so high it almost completely covers the ground, also causing shade. F. A closer look reveals that withered *Empetrum* leaves are common. Photographs were taken in the Varanger Peninsula National Park in July 2015 by last author.
Figure S3 Comparison between Ifjord and other regions within Northern Fennoscandia. Comparison of Empetrum abundance (A), green leaf bioactivity (B), chemical content of Batatasin-III (C) and total Phenols (D) from Ifjord, where the snowbed study was conducted, compared to that of other regions within Northern Fennoscandia. Boxplots display outliers when more than 1.5 times the interquartile range.

Appendix 1. Leaf traits enabling dispersal

*Empetrum nigrum* has small leaves (Lid & Lid 2005). Small size is a leaf trait that is likely to facilitate dispersal. In order to compare leaf traits of *Empetrum* to that of seed traits with known dispersal range, we measured 50 *Empetrum nigrum* fresh leaves. The leaves had an average leaf weight of 0.53 mg (+/- 0.11 mg), with an average length between 4.5 to 5 mm and a width of 1 mm at the broadest point. Comparing these values to that of the terminal velocity of wind dispersed seeds of similar proportions (Kattge *et al.* 2011), suggests *Empetrum* leaves have aerodynamic properties that facilitate wind dispersal. In addition, these leaf trait values suggest *Empetrum* leaves are easily transported with meltwater streams (Körner 2003).

Appendix 2. Pre-experiment assessment of bioactivity of leaves

The bioactivity of the collected withered leaves was assessed in a pre-experiment bioassay using lettuce (*Lactuca sativa*) seedlings. The bioassays were conducted using methodology as described in González *et al.* (2015). For each treatment 0 mg, 15 mg, 30 mg or 45 mg of withered leaves were placed into each of 15 Ø 4.5
cm Petri dishes with two pieces of Whatman No 1 filterpapers on top. The filterpapers were moistened with 1.5 ml distilled water before a set of 3 germinated lettuce seedlings were carefully transferred on top of the filterpaper and the lid attached with parafilm. The assay lasted for three days after which the rootlength of each seedling was measured and the average rootlength per petridish was calculated.

The withered *Empetrum* leaves collected for the allochthonous leaf treatment showed clear bioactivity at all treatment levels. The root lengths of the lettuce seedlings were reduced from an average of 2.933 cm of controls (no leaves) with -1.510, -2.071 and -2.373 cm (all ± 0.286 C.I.), at treatments with 15, 30 and 45 mg withered leaves per Petri dish respectively.

**Figure S4.** Root length of lettuce seedlings in response to a three-day treatment with allochthonous (withered) leaves at concentrations of 0, 15, 30 and 45 mg leaves per Petri dish.

Using the molecular weight of Batatasin-III of 244.28 g/mol (molecular formula of Batatasin-III in SI I) and anticipating a leaf concentration of 5 mg Batatasin-III g⁻¹ allochthonous leaves (Figure 5 this study), the 0, 15, 30 and 45 mg leaf treatments in the experiment correspond to a Batatasin-III treatment effect of 0, 0.3, 0.6 and 0.9 µMol respectively. This allochthonous treatment effect is a magnitude lower than the 0.1 - 4 mM applied in bioassays finding clear effects of Batatasin-III (González *et al.* 2015). The leaf treatments are however more similar to the concentration of 1.3 µM Batatasin-III, as calculated from a concentration of 0.32 µg Batatasin-III g⁻¹ humus collected at *Empetrum* sites (Bråthen, Fodstad & Gallet 2010). This latter concentration was also related to a very clear autochthonous allelopathic effect, significantly reducing the growth of the grass *Avenella flexuosa* and the forb *Solidago virgaurea*, two of the species also tested here, during a nine-week long experiment (Bråthen *et al.* 2010). Importantly therefore, it may not be Batatasin-III alone that cause reduced growth as *Empetrum* leaves of low or no Batatasin-III content still have high bioactivity (González *et al.* 2015).
Appendix 3. Baseline germination rate

In order to obtain a baseline germination rate, 100 seeds of each species were placed in a 4.5 cm diameter petri dish equipped with Whatmann filterpaper (Nr. 1), moistened with 1.5 ml distilled water and germinated at room temperature. Germination rates differed markedly between the species: Bistorta vivipara (94.67 %), Omalotheca supina (57.12 %), Pedicularis lapponica (0 %), Sibbaldia procumbens (52.34 %), Solidago virgaurea (78.11 %), Anthoxantum nipponicum (73.98 %), Avenella flexuosa (60.34 %), Nardus stricta (63 %), Chamaepericlymenum suecicum (0 %), Dryas octopetala (89.47 %), Empetrum nigrum (2.47 %), Salix herbacea (89.79 %), and Vaccinium myrtillus (76.80 %).

Appendix 4. Soil analysis

In order to compare the make-up of soils from the five sampling locations, soil analyses were conducted. A total of three samples of 25 gram soil from each of the five soil sampling sites were taken for chemical analysis of pH and content of P, K and N, whereas a total of ten samples of 5 gram soil were taken for a combustion analysis of water and C content. Chemical analysis was conducted at the Institute for Sustainable Plant Production (NPP) of the Austrian Agency for Health and Food Safety (AGES). The combustion analysis was conducted at UiT. Each 5 gram sample of soil was placed in a pre-weighed crucible. The samples were then dried at 95 degrees Celsius for 12 hours and allowed to cool before weighing. Thereafter, the samples were incinerated at 450°C for 4 hours, allowed to cool and then weighed again before the soil organic, ash and water content were calculated. Water was calculated as the weight of the dried sample subtracted from the weight of the fresh sample, the organic Carbon content as the weight of burnt sample subtracted from the weight of the dried sample, and the inorganic Carbon content (ash) as the weight of the burnt sample.

Table S1. Soil content description for each of the five sites included in the greenhouse experiment.

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