Title

Circum-Arctic distribution of chemical anti-herbivore compounds suggests biome-wide trade-off in defence strategies in arctic shrubs

Abstract and keywords

Spatial variation in plant chemical defence towards herbivores can help us understand variation in herbivore top-down control of shrubs in the Arctic and possibly also shrub responses to global warming. Less defended, non-resinous shrubs could be more influenced by herbivores than more defended, resinous shrubs. However, sparse field measurements limit our current understanding of how much of the circum-Arctic variation in defence compounds is explained by taxa or defence functional groups (resinous/non-resinous). We measured circum-Arctic chemical defence and leaf digestibility in resinous (*Betula glandulosa*, *B. nana* ssp. *exilis*) and non-resinous (*B. nana* ssp. *nana*, *B. pumila*) shrub birches to see how it varies among and within taxa and functional groups. Using LC-MS metabolomic analyses and *in-vitro* leaf digestibility via incubation in cattle rumen fluid, we analysed defence composition and leaf digestibility in 128 samples from 44 tundra locations.

We found biogeographical patterns in anti-herbivore defence where mean leaf triterpene concentrations and twig resin gland density were greater in resinous taxa and mean concentrations of condensing tannins were greater in non-resinous taxa. This indicates a biome-wide trade-off between triterpene or tannin dominated defences. However, we also found variations in chemical defence composition and resin gland density both within and among functional groups (resinous/non-resinous) and taxa, suggesting these categorisations only partly predict chemical herbivore defence. Complex tannins were the only defence compounds negatively related to *In-Vitro* Digestibility, identifying this previously neglected tannin group as having a potential key role in birch anti-herbivore defence.

We conclude that circum-Arctic variation in birch anti-herbivore defence can be partly derived from biogeographical distributions of birch taxa, although our detailed mapping of plant defence provides more information on this variation and can be used for better predictions of herbivore effects on arctic vegetation.

Keywords

Plant chemical defence, shrubs, birch, Betula, herbivory, tundra, Arctic, metabolomics

Introduction

In the absence of trees, the tallest vascular plants in the tundra are shrubs. They are currently increasing in abundance, height and distribution across the Arctic, and global warming is a main driver of these changes (e.g. Myers-Smith et al., 2011; Bjorkman et al., 2018; Berner et al., 2020). This increase in shrubs may lead to a decline in plant diversity (Myers-Smith et al., 2011), decreased albedo (Sturm et al., 2005), and increased greenhouse gas emissions (Hartley et al., 2012) in tundra ecosystems. Arctic shrubification patterns are, however, highly variable across space and time and sometimes deviate from observed warming trends (Berner et al., 2020). A factor that can contribute to this variability is herbivory (Christie et al., 2015). Large herbivores such as caribou/reindeer, sheep and muskoxen already slow down shrub expansion and growth in many arctic ecosystems (e.g. Post & Pedersen, 2008; Olofsson et al., 2009; Hofgaard et al., 2010; Bråthen et al., 2017), but not all (Tremblay et al., 2012). Insect herbivores can also cause severe defoliation and suppress woody plants during mass outbreak events (Jepsen et al., 2008; Prendin et al. 2019), but such events are rare in the tundra where even background insect herbivory is low (Barrio et al., 2017). How efficiently herbivores may limit arctic shrub expansion depends on shrub palatability and digestibility. This is partly linked to their growth rates and resource acquisition strategies since more rapidly growing plants generally have nutrient rich and thin (high specific leaf area) leaves (Wright et al., 2004). All shrubs also produce chemical anti-herbivore defence compounds (Kramer & Kozlowski, 1979), with presumed deterrent and/or digestion inhibiting effects on herbivores (Bryant et al., 1991; Christie et al., 2015).

Deciduous shrubs of the genus *Betula* (hereafter shrub birches or birches) are common in most vegetated areas in low arctic tundra and are often subject for ecological studies on shrub response to climate change. Their most important chemical defences against herbivores are suggested to be carbon-based triterpenes and condensed tannins (Bryant et al. 2014). At least some triterpene compounds are directly toxic to mammalian herbivores (McLean et al., 2009), while condensed tannins should primarily reduce plant nutritional quality by inhibiting protein digestion and are thus often categorised as digestive reducers (Julkunen-Tiitto et al., 1996). Other compounds present in birch (Bryant et al. 2014) and often considered in herbivore defence studies, are hydrolysable tannins that can cause oxidative stress in herbivores (Barbehenn, et al., 2006) and flavonoids that can act as antifeedants but mainly protect plants from oxidative stress and solar radiation (Kumar & Pandey, 2013). Even within each compound group, there is a large diversity of different metabolites that can act as deterrents separately and/or have co-active effects (Gershenzon et al., 2012).

Defence compound composition can differ within species (Christie et al., 2015), as in the dwarf-birch *Betula nana* that includes subspecies that are either mainly terpene-defended (*Betula nana* ssp. *exilis*) or mainly tannin-defended (*Betula nana* ssp. *nana*). From a chemical plant defence perspective, these defence systems define two functional groups; resinous birches (e.g. *B. nana* ssp. *exilis*) and non-resinous birches (e.g. *B. nana* ssp. *nana*). Resinous birches are often considered to have a strong chemical defence, with twigs densely covered with resin glands that produce a resin rich in toxic dammarane triterpenes (McLean et al., 2009). These triterpenes show antifeeding effects on snowshoe hare and muskoxen when added to otherwise palatable food (Reichardt et al., 1984; White & Lawler, 2002). Non-resinous birches, on the other hand, should lack the triterpene-rich resin and instead be predominantly defended by condensed tannins (Julkunen-Tiitto et al., 1996; Graglia et al., 2001). Condensed tannins are often presumed as weaker defence compounds compared to triterpenes and non-resinous birches are therefore presumed as more palatable to vertebrate herbivores than resinous birches (Bryant et al. 2014). A study directly comparing *B. nana* ssp. *nana* and *B. nana* ssp. *exilis* from two high latitude sites suggests that non-resinous birches compensate for their lack of triterpenes by investing more carbon in greater production of tannins (Graglia et al., 2001). The generality of this trade-off across the Arctic is not known but if it is present at larger spatial scales, this trade-off between triterpene and tannin defences is important for understanding how palatability of shrub birches varies across the Arctic and, by extension, to which degree their abundance and distribution may be limited by herbivory.

Bryant et al. (2014) proposed the hypothesis that herbivores may have a stronger effect on shrub birch abundance in areas with non-resinous birches (Fennoscandia, Iceland, Greenland, and West Siberia) compared to areas dominated by resinous birches (most of the Canadian Arctic, Alaska, and East Siberia), owing to their differences in chemical defence. Geographic variation in chemical defence could therefore be a major factor driving differences in top-down control of shrub birches by herbivores, and thus potentially explain variation in warming-driven shrubification patterns and greening across the Arctic (Bryant et al., 2014). Sparse field measurements currently limit our possibility to properly estimate how much of the circum-Arctic variation in anti-herbivore defence is actually explained by taxa or functional groups. Given that polyphenolic defence compounds can vary greatly at small spatial scales, between phenological phases (Torp et al., 2010) and over time (Salminen et al., 2002) within the same taxa, there is cause to believe that generalisations of chemical defence within taxa or functional groups might not be completely straightforward. More large-scale data measured across the climatic, geological and biotic gradients in arctic tundra using comparable methods are needed to address this.

To test to what extent circum-Arctic variation in chemical defence composition in resinous and non-resinous birches can be explained by plant taxa and whether there is a trade-off between triterpene and tannin defence, we mapped the variation in chemical anti-herbivore defence in tundra shrub birches. We sampled resinous (*Betula nana* ssp. *exilis*, *B. glandulosa*) and non-resinous (*B. nana* ssp. *nana*, *B. pumila*) shrub birches at 128 sampling sites within 44 locations across the circumpolar Arctic, and analysed concentrations of chemical defence compounds using LC-MS metabolomics analysis. To determine an aspect of the importance of these defence compounds for vertebrate herbivores we also tested their effect on *in-vitro* leaf digestibility. We hypothesise that: *(I) Defence compound composition in tundra shrub birches shows more variation across the circumpolar Arctic than can be predicted based on taxa or functional group (resinous or non-resinous) alone. (II) There is a trade-off between triterpenes and tannins in shrub birches that can be detected at circum-Arctic scale, where non-resinous birches produce higher concentrations of tannins compared to triterpene-defended resinous birches that instead produce more triterpenes. (III) Tannins reduce leaf digestibility to a greater extent than triterpenes that deter herbivores through other mechanisms.*

Methods

Study area and sampling

Our study covers circumpolar tundra vegetation in the Northern Hemisphere, at latitudes between 47.3°N and 74.5°N (Fig. 1), where sites (area of ~10 m radius) were selected based on shrub birch presence. During June-August 2014, we sampled resinous (*Betula glandulosa, Betula nana* ssp. *exilis*) and non-resinous dwarf birch (*Betula nana* ssp. *nana*, *Betula pumila*) at 128 sampling sites within 44 locations (Table S1). Evenly distributed across each site, we randomly chose 10 individuals and sampled 50 random short-shoot leaves (leaf rosettes along the stem with leaves of the same age; n=500 per site), and 10 random long-shoots (twigs and leaves representing the current annual growth; n=100 per site) from each individual. All samples were air-dried in the field and further oven-dried at 60°C for 48 hours upon arrival to the lab. We used the short-shoot leaves for chemical analyses to achieve as much phenological homogeneity as possible among samples and long shoot twigs for resin gland counts. Short-shoot leaf samples from the same site were pooled and ground with a ball mill prior to chemical analyses of defence compounds, total nitrogen (N), carbon (C) and *In-Vitro* Digestibility.

Herbivores

The main large herbivores across our sites are reindeer or wild caribou (*Rangifer* sp.) with the exception of Zackenberg in eastern Greenland where muskoxen are the main herbivores and Audkuluheidi on Iceland where mainly sheep graze. Muskoxen also occur in other parts of Greenland, North America and Siberia and moose might visit sites close to the treeline. Insect herbivores exist across the whole tundra, but none of our samples were taken in sites with active outbreaks.

Metabolite profiling

To profile chemical compounds connected to plant anti-browsing defence in tundra dwarf birch, we performed an untargeted metabolomics analysis by liquid chromatography - mass spectrometry (LC-MS) at the Swedish Metabolomics Centre, Umeå University. We extracted 10 mg of ground leaf material, according to Gullberg et al. (2004), and analysed 2 µl of the extracts according to Abreu et al. (2020). Compounds were detected with an Agilent 6550 Q-TOF mass spectrometer with an electrospray ion source operating in negative ion mode. The MS files were processed by a targeted feature extraction using several in house phenolic databases. Ionized mass from triterpenes was manually scanned across the MS spectra. When the same ion was present in different retention time (e.g. due do in source fragmentation) it was annotated as ‘derivative’. Metabolite identification was based on the diagnostic fragments produced during LC-MS analysis.

Defence compound groups

We identified about 100 metabolites (Table S2), including several classes of defence compounds previously reported to contribute to plant anti-browsing defence, such as triterpenes, condensed tannins, hydrolysable tannins, flavonoids and chlorogenic acid as well as complex tannins. Complex tannins build up from a condensed tannin unit and a hydrolysable tannin unit, but are rarely considered or studied in ecological research. It is reasonable to assume complex tannins have similar protein precipitating properties as condensed tannins since they contain condensed tannin units. Flavonoids and chlorogenic acid (a precursor to phenolic compounds) are often considered of low importance as antifeedants (Kumar & Pandey, 2013), therefore they are mainly presented in the supplementary information of this study.

Resin gland density

To measure resin gland density, we defoliated 10 long-shoot twigs of each sample, photographed 15 mm twig segments starting 20 mm from the twig top and counted all visible resin glands. We measured the twig diameter at the beginning and end of all segments to calculate the gland count area (approximated to a half of the total segment bark area). We then calculated gland/mm^2 and used this as a standardised measure of resin gland density.

In-Vitro Digestibility (IVOMD)

We measured general sample digestibility as *in-vitro* organic matter digestibility (hereafter *In-Vitro* Digestibility or IVOMD). Dry leaf material was mixed with rumen fluid from dairy cows, incubated at 38°C for 96 hours and the proportion of digested organic matter was then calculated, all according to Lindgren (1979). Although digestibility can be herbivore species specific to some extent, overall differences in digestibility of food plants to different grazers and browsers can be estimated by *in-vitro* organic matter digestibility with rumen fluid from dairy cows (Krizsan et al., 2018). The analyses were done at HUV Analysis Laboratory, Swedish University of Agricultural Sciences, Uppsala.

Proxy of Specific Leaf Area (SLA-P)

For all sampling sites, we weighed and scanned 10 random leaves from the pooled sample, and calculated the leaf area of dry leaves using the ROI manager tool in ImageJ software (Schneider et al., 2012). We used both long- and short-shoot leaves to standardise for fully developed leaves representing the whole birch individuals. Since our samples were dried upon arrival, we estimated an SLA-Proxy (our abbreviation; SLA-P) as [dry leaf area/dry mass] instead of SLA as [fresh leaf area/dry mass]. The results are to be found in supplementary material (Fig. S1).

Nitrogen and carbon

Ground leaf samples were analysed for total nitrogen (N) and carbon (C) concentrations on an elemental analyser interfaced to a continuous flow isotope ratio mass spectrometer (IRMS, UC Davis Stable Isotope Facility, University of California).

Climate

To relate the shrub taxa and their chemical defence composition to local climate conditions, we collected interpolated grid data with a resolution of 30 arc-seconds (~1 km) on annual precipitation, mean annual temperature, winter temperature (mean temperature of January) and summer temperature (mean temperature of July) from the WorldClim 1.4 database (Hijmans et al., 2005). Climate data for each sampling location were extracted using GIS software ArcMAP version 10.4.1. (ESRI, 2011). We present these relationships in the supplementary material (Fig. S2).

Statistical analyses

The chemical defence compounds were classified into six different groups of compounds; triterpenes, condensed tannins, hydrolysable tannins, complex tannins, flavonoids, and chlorogenic acid (precursor to phenolic compounds). We included all compound groups for the statistical analyses to capture the overall diversity of chemical defence composition (hypothesis I), but since triterpenes and tannins have the strongest contribution to plant defence we focus mainly on these in the main results (see SI for additional information on flavonoids and chlorogenic acid). Similarities and differences in the chemical anti-herbivore defence composition across tundra shrub birches were explored by Non-metric Multidimensional Scaling Analysis (NMDS, Minchin, 1987) using the 'metaMDS' function of the 'vegan' package (Oksanen et al., 2019). In the NMDS we included all detected separate secondary metabolites from all six compound groups. To see if chemical defence composition relates to other defence associated attributes, we fitted resin gland density, leaf digestibility and leaf N and C content to the NMDS using the 'envfit' function in the 'vegan' package. Climatic and temporal associations to chemical composition was also tested with the 'envfit' function and is presented in supplementary material (Fig. S2). Additionally, we examined similarities and dissimilarities in chemical defence among the shrub birches using a cluster analysis with euclidean distance and Ward's minimum variance clustering method ('hclust' function in the 'stats' package), data were mean centred and scaled by standard deviation per sample. We used one-way ANOVAs to test for intertaxon differences in chemical defence and other plant traits. The chemical defence was tested on compound group level, so for this we summed all compounds within each of the six groups and tested for differences among species. When we assessed the relationship between triterpenes and tannins (hypothesis II), we included both condensed tannins and complex tannins in the tannin group given that complex tannins also consist of condensed tannin units. Hydrolysable tannins do not have any condensing effects and are therefore not included in these analyses. We tested the relationship between tannin and triterpene concentrations with a linear regression. We also used linear regressions to test the relationship between concentrations of the most important defence compounds (triterpenes, condensed, hydrolysable and complex tannins) and *In-Vitro* Digestibility (hypothesis III). For the significant relations, we examined taxon dependencies using additional two-way ANOVAs. All statistical analyses were performed using R software (version 4.0.3) (R Development Core Team, 2020).

Results

We found clear large-scale patterns in spatial distribution in three out of the four most important defence compound groups (triterpenes, condensed tannins, hydrolysable tannins, complex tannins; Fig. 1), but also circum-Arctic variation in defence compound concentration in tundra shrub birch leaves.

Triterpene concentrations were highest in Eastern Siberia and most of North America, and very low in eastern Canada (island of Newfoundland), Greenland, Iceland, Scandinavia and Western Siberia (Fig. 1a). We found the opposite pattern for condensed tannin and complex tannin concentrations (Fig. 1b, c). Hydrolysable tannin concentrations varied less among biogeographical regions, except that we found the lowest concentrations of these compounds in Newfoundland, eastern Canada (Fig. 1d).

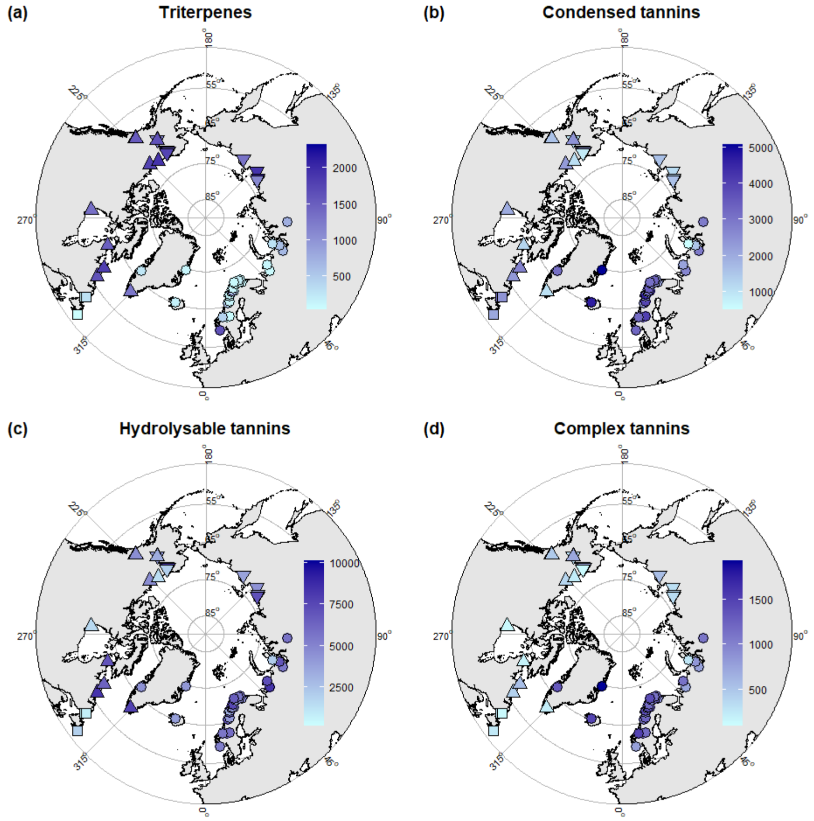

Figure 1. Maps showing relative concentrations [Peak area/mg*10^{-4} leaf tissue] of chemical compounds involved in the anti-browsing defence in tundra shrub birches. The maps cover high (dark blue) and low (light blue) concentrations of four compound classes; (a) triterpenes, (b) condensed tannins, (c) hydrolysable tannins and (d) complex tannins, in four tundra shrub birch taxa; *Betula glandulosa* (up-facing triangles), *B. nana* ssp. *exilis* (down-facing triangles), *B. nana* ssp. *nana* (circles) and *B. pumila* (squares).

In the two-dimensional NMDS analysis (stress level=0.09), higher scores on the x-axis (NMDS1) were related to increasing tannin and decreasing terpene concentrations, while the second axis was mainly a gradient in condensed to hydrolysable tannins with higher condensed and lower hydrolysable tannin concentrations at high NMDS2 scores (Fig. 2). NMDS1 was negatively associated with higher resin gland density and *In-Vitro* Digestibility (IVOMD) (Fig. 2). The ranges of NMDS scores for each taxon showed that the non-resinous birches *B. pumila* and *B. nana* ssp. *nana* were clearly separated both from each other and the resinous taxa (*B. nana* ssp. *exilis* and *B. glandulosa*), while the resinous birches had highly overlapping chemical composition. The IVOMD of birch leaves was negatively related to complex tannins ($p=0.003$, $R^2=0.07$), and positively related to triterpene ($p<0.001$, $R^2=0.19$) and hydrolysable tannin ($p=0.02$, $R^2=0.04$) concentration (Fig. 3a, c-d). IVOMD was however not related to condensed tannin concentrations (Fig. 3b). For the significant relations between IVOMD and separate compound group concentrations, we found intrataxon variation for triterpenes and complex tannins as well as both intra- and intertaxon variation for hydrolysable tannins. An additional cluster analysis revealed three main groupings across the taxa based on chemical defence composition with *B. nana* ssp. *nana* as one group, *B. nana* ssp. *exilis* and most *B. glandulosa* as a second group and lastly a third group with non-resinous *B. pumila* and a few resinous *B. glandulosa* samples (Fig. 2). More details on chemical similarities/differences among samples can be found in supplementary material (Fig. S3).

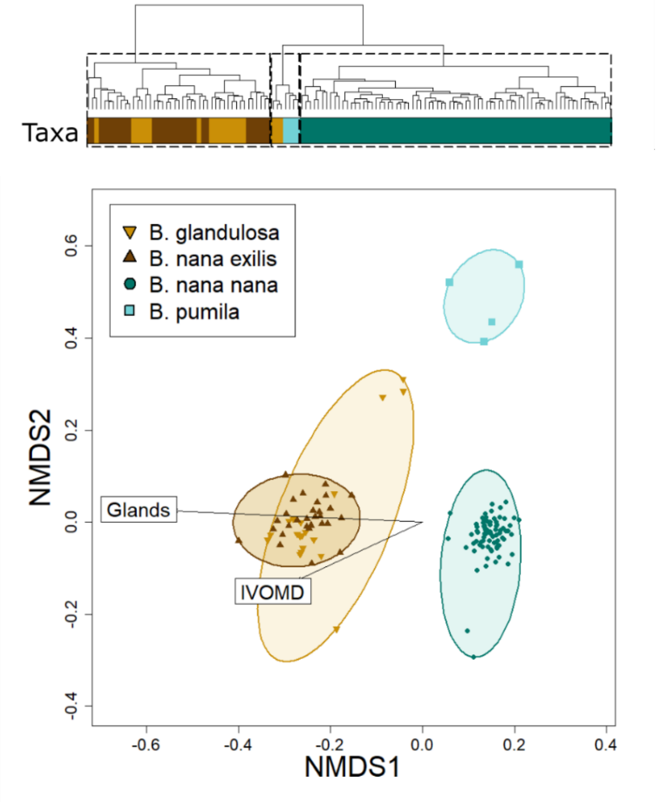

Figure 2. Non-Metric Dimensional Scaling (NMDS) ordination and cluster diagram that show differences and similarities in chemical defence composition in resinous birches, *Betula glandulosa* (beige down-facing triangles) and *B. nana* ssp. *exilis* (brown up-facing triangles), and non-resinous birches, *B. nana* ssp. *nana* (dark blue circles) and *B. pumila* (light blue squares). In the NMDS, ellipses represent the data range for each taxon and black arrows show strength and direction of significant relationships between NMDS1 and resin gland density and *In-Vitro* Digestibility. The coupled cluster diagram shows the three main clusters (in boxes) that form across the taxa based on chemical defence composition.

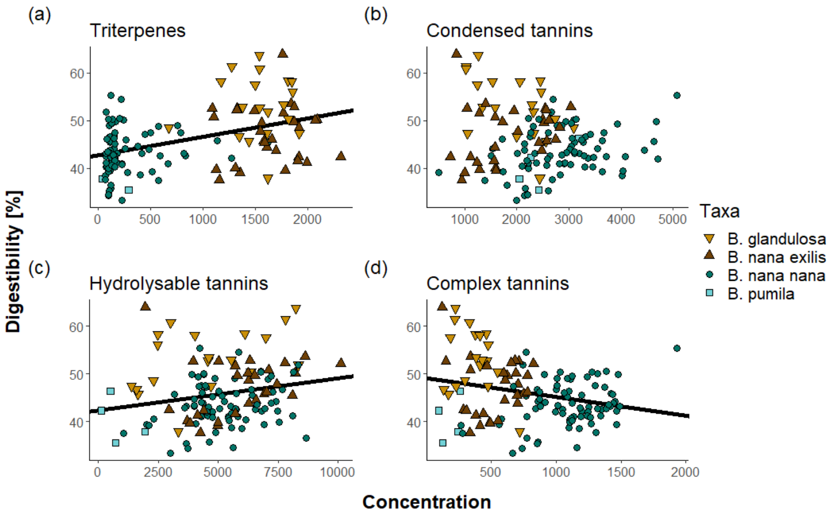

Figure 3. Linear relationships between digestibility (*In-Vitro* Organic Matter Digestibility; [%] meltability of organic dry matter in cow rumen) and chemical defence compound concentration ([Peak area/mg*10^{-4}] in triterpenes, condensed, hydrolysable and complex tannins) in four tundra shrub birch taxa; resinous *Betula glandulosa* (beige) and *B. nana* ssp. *exilis* (brown), and non-resinous *B. nana* ssp. *nana* (dark blue) and *B. pumila* (light blue). Significant linear relationships are drawn with bold lines.

The most apparent difference between the four birch taxa was higher triterpene concentration in resinous birches, *B. glandulosa* and *B. nana* ssp. *exilis*, compared to non-resinous birches, *B. nana* ssp. *nana* and *B. pumila* (Table 1; Fig. 4a). Additionally, *B. nana* ssp. *nana* had a higher condensed tannin concentration than the two resinous taxa, *B. glandulosa* and *B. nana* ssp. *exilis*, while condensed tannin concentrations in *B. pumila* did not differ from the other taxa (Table 1; Fig. 4b). Hydrolysable tannin concentrations were higher in *B. nana* ssp. *exilis* than in *B. glandulosa*, while concentrations in *B. nana* ssp. *nana* were intermediate, and did not differ from the two resinous taxa (Table 1; Fig. 4c). In *B. pumila,* hydrolysable tannin concentrations were much lower than in any other taxa (Table 1; Fig. 4c). The complex tannin concentration was higher in *B. nana* ssp. *nana* compared to the other three taxa, and lower in *B. pumila* compared to the two *B. nana* subspecies (Table 1; Fig. 4d). Flavonoid concentration was highest in *B. nana* ssp. *nana* and lowest in *B. pumila* (Table 1; Fig. S4a), and there were no differences in chlorogenic acid concentrations between the four taxa (Table 1; Fig. S4b). In general, the variation within taxa was large for all compounds (even triterpenes), resulting in overlapping concentrations among taxa. Across all samples, triterpene concentration was negatively correlated to concentrations of condensed and complex tannins combined, which indicate a compensatory production of these tannins in shrubs that lack triterpenes ($p<0.001$, $r^2=0.29$) (Fig. 4e).

Table 1. One-way ANOVAs testing interspecific variation in chemical compounds and other attributes involved in plant anti-herbivore defence in four tundra shrub birch taxa (*B. glandulosa*, *B. nana* ssp. *nana*, *B nana* ssp. *exilis*, *B. pumila*). Significant values are written in bold.

	Taxon variation		
Source of variation	*Df*	*F*	*P*
Triterpene	**3**	**200.1**	**<0.001**
Condensed tannin	**3**	**15.75**	**<0.001**
Hydrolysable tannin	**3**	**11.03**	**<0.001**
Complex tannin	**3**	**73.14**	**<0.001**
Flavonoids	**3**	**66.25**	**<0.001**
Chlorogenic acid	3	1.05	0.373
Gland density	**3**	**232**	**<0.001**
In-Vitro Organic Matter Digestibility (IVOMD)	**3**	**3.18**	**0.027**
Carbon	3	0.76	0.517
Nitrogen	**3**	**2.97**	**0.034**
Residuals	*124*[a]		

[a] For all but 'Gland density' with residuals = 123

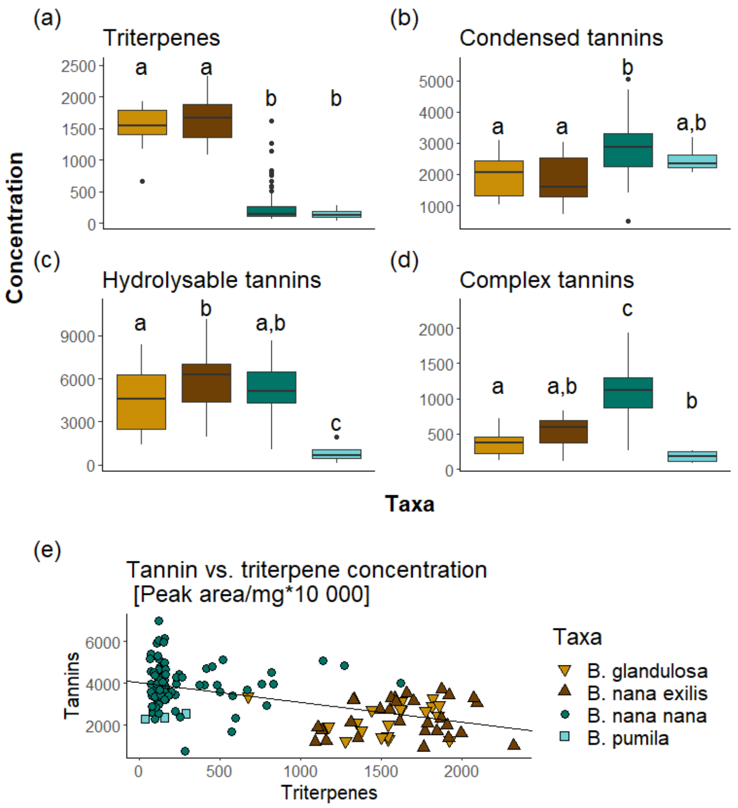

Figure 4. Boxplots showing (a) triterpene, (b) condensed tannin, (c) hydrolysable tannin and (d) complex tannin concentrations [Peak area/mg*10^{-4}] in resinous, *Betula glandulosa* (beige, n=19) and *B. nana* ssp. *exilis* (brown, n=33), and non-resinous, *B. nana* ssp. *nana* (dark blue, n=76) and *B. pumila* (light blue, n=4), tundra shrub birches. Boxplots show median (thick line), interquartile range (box), outer quartile (error bar) and outliers outside two interquartile ranges (dots). Significant differences in group means are annotated by lowercase letters. Scatterplot E) shows the significant linear relationship between tannin (condensed + complex) and triterpene concentration for all four birch taxa.

As expected, twig resin gland density was higher in the resinous taxa *B. glandulosa* and *B. nana* ssp. *exilis* than in the non-resinous taxa *B. nana* ssp. *nana* and *B. pumila*, this was clearly distinct and gland density values do not overlap between the two functional groups (Table 1; Fig. 5a). IVOMD was highest in *B. glandulosa*, intermediate in *B. nana* ssp. *exilis* and *B. nana* ssp. *nana*, and lowest in *B. pumila*, but the differences between *B. pumila* and the two *B. nana* subspecies were not statistically significant (Table 1, Fig. 5b). N concentrations were similar among the four taxa and even though it tended to be slightly higher in *B. nana* ssp. *exilis* than in *B. glandulosa* (Table 1, Fig. S5a) it did not explain any of the differences in defence composition (Fig. 2). Also, there was no difference in C concentration among the four taxa (Table 1, Fig. S5b).

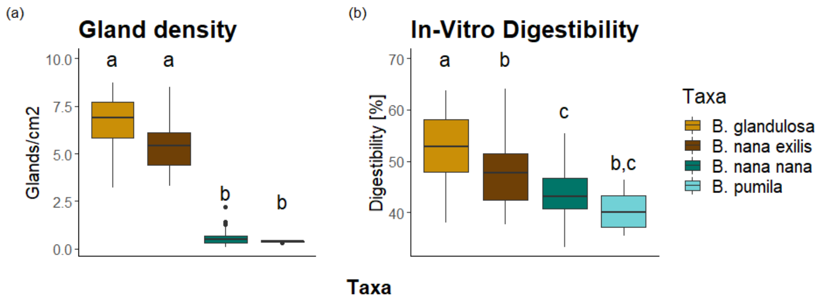

Figure 5. Boxplots showing twig resin gland density (a) and leaf *In-Vitro* Digestibility (b) in resinous, *Betula glandulosa* (beige, n=19) and *B. nana* ssp. *exilis* (brown, n=33), and non-resinous, *B. nana* ssp. *nana* (dark blue, n=76) and *B. pumila* (light blue, n=4), tundra shrub birches. The boxplots show median (thick line), interquartile (box), outer quartile (error bar) and outliers outside two interquartile ranges (dots). Significant differences in group means are annotated by lowercase letters.

Discussion

Our spatially and chemically detailed data on anti-herbivore defence in birch leaves reveal biogeographic patterns but also considerable circum-Arctic variation in chemical defence within and among different taxa of tundra shrub birches. As hypothesised, leaves from resinous birches (*B. nana* ssp. *exilis*, *B. glandulosa*) had higher triterpene concentrations than leaves from non-resinous taxa (*B. nana* ssp. *nana, B. pumila*). This is expected since triterpenes are mostly produced in the resin glands (Bryant et al., 2014), and occurrence of resin glands is one of the characteristics separating resinous and non-resinous birches from each other (Flora of North America, 2008). Nevertheless, we also found support for hypothesis (I); that variation in chemical defence composition across the Arctic is not fully explained by functional groups or taxa. In general, we found the highest triterpene concentrations in the birch taxa with high resin gland density although the differences in triterpene concentrations were not as pronounced as differences in resin glands on twigs. We also found a few non-resinous *B. nana* spp. *nana* samples that had relatively high leaf triterpene concentrations despite low gland density, suggesting that leaf triterpene concentrations are not always directly associated with the number of resin glands on twigs. Non-resinous birches can have a few resin-producing glands (Bryant et al., 2014), which in some cases in our study resulted in triterpene concentrations similar to some resinous birches. We also reveal that despite being densely covered by glands, resinous birches (*B. glandulosa*) may lack triterpenes almost entirely and instead have a chemical defence composition that is similar to the non-resinous and poorly gland-covered *B. pumila* (Fig. S4). These findings indicate substantial variability in chemical defence by triterpenes that can be unrelated to resin gland density and result in overlaps in triterpene concentration among resinous and non-resinous taxa. Thus, morphological characters such as resin glands are not alone sufficient to predict herbivore defence in leaves and the geographical distribution of resinous/non-resinous birches cannot adequately predict to what extent shrubs will be retarded by herbivores.

In agreement with our second hypothesis (II), non-resinous birch compensate for their lower concentrations of triterpenes by producing more condensing tannins, but not only by higher condensed tannin production but also higher complex tannin production. Specifically, *B. nana* ssp. *nana* has the highest concentrations of both condensed and complex tannins of all birch taxa in our study. Compared to its resinous counterpart, *B. nana* ssp. *exilis*, condensed tannin concentration is more than 50% greater, and complex tannin concentration more than twice as great, in *B. nana* ssp. *nana*. Our results confirm findings of condensed tannin concentrations being up to 50% higher in non-resinous *B. nana* ssp. *nana* from Abisko, Sweden, compared to resinous *B. nana* ssp. *exilis* from Toolik, Alaska (Graglia et al. 2001), and show that this pattern may be widespread across the Arctic. We also found differences in tannin composition between the non-resinous birches. *Betula pumila* are almost exclusively defended by condensed tannins, while *B. nana* ssp. *nana* is characterised by a more diverse defence system which also includes hydrolysable and complex tannins. Within the resinous birch taxon (*B. glandulosa* and *B. nana* ssp. *exilis*) we also found substantial variation in tannin composition, even though these two taxa, categorised as different species, cannot be separated based on their defence composition. Also, despite sampling date did not influence plant defence concentrations in our study (Fig. S2), temporal patterns are common for defence compound concentrations (Salminen et al., 2002). These findings emphasize that both non-resinous and resinous birch taxa can vary greatly in tannin composition across the Arctic, further indicating challenges for circum-Arctic generalisations of chemical defence composition.

Mapping the concentrations of plant defence across the Arctic is essential for understanding how effects of trophic interactions and climate change on plant growth varies across the Arctic (Christie et al., 2015). Shrub birches are especially important in the Arctic because they are dominant in many parts of the tundra biome, respond quickly to warming and fertilization (Chapin et al., 1995) yet can be affected by herbivory (Olofsson et al., 2009). Both triterpenes and tannins are complex compound groups, but still often suggested as deterrents of both vertebrate and insect herbivory (Barbehenn, *et al*., 2011; Christie et al., 2015). The inhibitory functions of triterpenes on herbivores are diverse (Pichersky & Raguso, 2018). They can, for example, be directly toxic by influencing the nervous system or by acting as hormone analogues in herbivores (Agrawal et al., 2012). Tannins are a diverse group of polyphenolics, and their effects on herbivores are even more variable (Salminen & Karonen, 2011). Most tannins have the potential to bind and precipitate proteins (Marsch *et al*., 2020), but condensed tannins are the ones presumed to have the highest capacity to reduce protein digestion (Jayanegara & Palupi, 2010). The effects of hydrolysable tannins are even less clear, but at least some of them reduce food digestibility by complex-binding sugar molecules (Goel et al., 2005) and some cause oxidative stress in herbivores (Barbehenn, et al., 2011).

Our test of plant digestibility at least partly confirmed hypothesis (III), that tannins reduce leaf digestibility while triterpenes do not since they deter herbivores through other mechanisms. Only one tannin group, complex tannins, showed a negative relationship to *In-Vitro* Digestibility in this study. This highlights the possible importance of these compounds in chemical anti-herbivore defence. There is essentially no data on the effect of complex tannins on herbivores, but since they are made up of both hydrolysable and condensed tannins, it makes sense that they act as digestion reducers, as indicated by this study. Although complex tannins have not been considered as a separate group in earlier ecological studies, they have probably been included in many of the traditional quantification methods of condensed tannins, like the acid-butanol (Porter et al., 1985) and vanillin assay (Price et al., 1978), since their condensed tannin unit is likely targeted by these methods. The lack of relationship between condensed tannins and digestibility in this study is thus not necessarily in conflict with previous studies that found a clear decreased leaf digestibility with higher condensed tannin concentrations (Gowda et al., 2019), since they have used traditional quantification methods likely targeting complex tannins as well. Hydrolysable tannins and triterpenes were actually positively related to leaf *In-Vitro* Digestibility. It is not likely that these compounds stimulate digestion, and we rather interpret the relationships as spurious correlations caused by negative correlations between triterpenes and hydrolysable tannins, and complex and condensed tannins. The leaf digestibility in our *in-vitro* inoculums in cow rumen ranged between 38-60%, which is consistent with earlier studies testing both *in-vitro* and *in-vivo* digestibility of *B. nana* ssp. *exilis* in caribou rumen (45 and 54 %, respectively) (Kuropat, 1984). Plant digestibility and palatability is, however, a product of complex plant syndromes (Agrawal & Fishbein, 2006), resulting from strong associations among a wide range of different chemical and morphological attributes, and it is therefore hard to disentangle the causal effects of single components in composite samples.

The mapping of plant defence compounds presented in this study can be an important tool for predicting how shrub birches defend themselves against herbivory across the Arctic and a prerequisite for future research. For example, the circum-Arctic range we find of the trade-off relation between triterpenes and condensed and complex tannins raises questions on the dose response of these compounds. Deriving effect equivalents or a shared defence currency among different compounds is needed to translate concentrations of different compounds to defence strength. Finding out the dose response of separate compound groups could also allow for estimates of relative costs for plants to produce certain types of defences and the cost for herbivores to cope with them, but data to do so are currently not available. Existing herbivore feeding trials do not fully resolve this question either since deterrent effects of different compound groups are generally tested separately (Reichardt et al., 1984; White & Lawler, 2002).

We are only aware of one study that compares relative palatability of non-resinous and resinous birches, which show that, in winter, free ranging snowshoe hares (Alaska) and mountain hares (Finland) feed preferentially upon the less resinous birch when presented with twigs from both birch types (Bryant, 1989). Since we in this study reveal that tannin and terpene concentrations vary substantially also within non-resinous and resinous birches, multisite feeding trials would be needed to test the generality of these results. Resinous birches are at least not totally avoided by herbivores since herbivores can have strong suppressing effects also on the resinous birch *B. glandulosa* (Manseau et al., 1996; Crête & Doucet, 1998; Andruko et al., 2020). Plant responsiveness to herbivory is not only a product of plant chemistry, but also depends on the ecological context in which plants grow. Herbivore density is an important aspect, which is often controlled to a greater extent by climate, landscape structure, predators and human activities than plant chemistry (Dahlgren et al., 2009, Skarin et al., 2020). Herbivore guild also matters since the same compounds might have different effects on different herbivores (Barbehenn et al., 2011). The extent to which plants are exposed to herbivory is also related to the composition and diversity of neighbouring plants since the quality of alternative resources can affect herbivore preference for certain plants (Barbosa et al., 2009) and herbivore attraction to certain areas (Palmer et al., 2003).

We conclude that although circum-Arctic variation in birch chemical defence can be partly explained by characterisation as taxon and defence type (resinous/non-resinous), there is also variation for which this is not the case. Detailed measurements of plant defence compounds, like ours, can then give a more accurate and complex description of the plant defence and will thus give us a more powerful tool to predict future vegetation patterns. While chemical analyses alone will not give a full understanding of plant-herbivore interactions, our circum-Arctic mapping of defence compounds can be a key contributor for understanding the strength of top-down control of shrub expansion in response to a warmer climate.

References:

Abreu, I.N., Johansson, A.I., Sokołowska, K., Niittylä, T., Sundberg, B., Hvidsten, T.R., Street, N.R. & Moritz, T. (2020) A metabolite roadmap of the wood-forming tissue in Populus tremula. *New Phytol.*, 228, 1559-1572. https://doi.org/10.1111/nph.16799

Agrawal, A.A. & Fishbein, M. (2006) Plant defense syndromes. *Ecology*, 87, 132–149. https://doi.org/10.1890/0012-9658(2006)87[132:pds]2.0.co;2

Agrawal, A.A., Petschenka, G., Bingham, R.A., Weber, M.G. & Rasmann, S. (2012) Toxic cardenolides: Chemical ecology and coevolution of specialized plant-herbivore interactions. *New Phytol.*, 194, 28–45. https://doi.org/10.1111/j.1469-8137.2011.04049.x

Andruko, R., Danby, R. & Grogan, P. (2020) Recent growth and expansion of birch shrubs across a low arctic landscape in continental Canada: Are these responses more a consequence of the severely declining caribou herd than of climate warming? *Ecosystems*, 23, 1362-1379. https://doi.org/10.1007/s10021-019-00474-7

Barbosa, P., Hines, J., Kaplan, I., Martinson, H., Szczepaniec, A. & Szendrei, Z. (2009) Associational Resistance and Associational Susceptibility: Having Right or Wrong Neighbors. *Annu. Rev. Ecol. Evol. Syst.,* 40, 1-20. https://doi.org/10.1146/annurev.ecolsys.110308.120242

Barbehenn, R.V & Constabel, C.P. (2011) Tannins in plant-herbivore interactions. *Phytochemistry*, 72, 1551-1565. https://doi.org/10.1016/j.phytochem.2011.01.040

Barrio, I.C., Lindén, E., te Beest, M., Olofsson, J., Rocha, A., Soininen, E.M., Alatalo, J.M., Andersson, T., Asmus, A., Boike, J., Bråthen, K.A., Bryant, J.P., Buchwal, A., Bueno, C.G., Christie, K.S., Denisova, Y.V., Egelkraut, D., Ehrich, D., Fishback, L.A., Forbes, B.C., Gartzia, M., Grogan, P., Hallinger, M., Heijmans, M.M.P.D., Hik, D.S., Hofgaard, A., Holmgren, M., Høye, T.T., Huebner, D.C., Jónsdóttir, I.S., Kaarlejärvi, E., Kumpula, T., Lange, C.Y.M.J.G., Lange, J., Lévesque, E., Limpens, J., Macias-Fauria, M., Myers-Smith, I., van Nieukerken, E.J., Normand, S., Post, E.S., Schmidt, N.M., Sitters, J., Skoracka, A., Sokolov, A., Sokolova, N., Speed, J.D.M., Street, L.E., Sundqvist, M.K., Suominen, O., Tananaev, N., Tremblay, J.-P., Urbanowicz, C., Uvarov, S.A., Watts, D., Wilmking, M., Wookey, P.A., Zimmermann, H.H., Zverev, V. & Kozlov, M.V. (2017) Background invertebrate herbivory on dwarf birch (Betula glandulosa-nana complex) increases with temperature and precipitation across the tundra biome. *Polar Biol.*, 40, 2265-2278. https://doi.org/10.1007/s00300-017-2139-7

Berner, L.T., Massey, R., Jantz, P., Forbes, B.C., Macias-Fauria, M., Myers-Smith, I., Kumpula, T., Gauthier, G., Andreu-Hayles, L., Gaglioti, B. v., Burns, P., Zetterberg, P., D'Arrigo, R. & Goetz, S.J. (2020) Summer warming explains widespread but not uniform greening in the Arctic tundra biome. *Nat. Commun.*, 11, 4621. https://doi.org/10.1038/s41467-020-18479-5

Bjorkman, A.D., Myers-Smith, I.H., Elmendorf, S.C., Normand, S., Rüger, N., Beck, P.S.A., Blach-Overgaard, A., Blok, D., Cornelissen, J.H.C., Forbes, B.C., Georges, D., Goetz, S.J., Guay, K.C., Henry, G.H.R., HilleRisLambers, J., Hollister, R.D., Karger, D.N., Kattge, J., Manning, P., Prevéy, J.S., Rixen, C., Schaepman-Strub, G., Thomas, H.J.D., Vellend, M., Wilmking, M., Wipf, S., Carbognani, M., Hermanutz, L., Lévesque, E., Molau, U., Petraglia, A., Soudzilovskaia, N.A., Spasojevic, M.J., Tomaselli, M., Vowles, T., Alatalo, J.M., Alexander, H.D., Anadon-Rosell, A., Angers-Blondin, S., Beest, M. te, Berner, L., Björk, R.G., Buchwal, A., Buras, A., Christie, K., Cooper, E.J., Dullinger, S., Elberling, B., Eskelinen, A., Frei, E.R., Grau, O., Grogan, P., Hallinger, M., Harper, K.A., Heijmans, M.M.P.D., Hudson, J., Hülber, K., Iturrate-Garcia, M., Iversen, C.M., Jaroszynska, F., Johnstone, J.F., Jørgensen, R.H., Kaarlejärvi, E., Klady, R., Kuleza, S., Kulonen, A., Lamarque, L.J., Lantz, T., Little, C.J., Speed, J.D.M., Michelsen, A., Milbau, A., Nabe-Nielsen, J., Nielsen, S.S., Ninot, J.M., Oberbauer, S.F., Olofsson, J., Onipchenko, V.G., Rumpf, S.B., Semenchuk, P., Shetti, R., Collier, L.S., Street, L.E., Suding, K.N., Tape, K.D., Trant, A., Treier, U.A., Tremblay, J.P., Tremblay, M., Venn, S., Weijers, S., Zamin, T., Boulanger-Lapointe, N., Gould, W.A., Hik, D.S., Hofgaard, A., Jónsdóttir, I.S., Jorgenson, J., Klein, J., Magnusson, B., Tweedie, C., Wookey, P.A., Bahn, M., Blonder, B., van Bodegom, P.M., Bond-Lamberty, B., Campetella, G., Cerabolini, B.E.L., Chapin, F.S., Cornwell, W.K., Craine, J., Dainese, M., de Vries, F.T., Díaz, S., Enquist, B.J., Green, W., Milla, R., Niinemets, Ü., Onoda, Y., Ordoñez, J.C., Ozinga, W.A., Penuelas, J., Poorter, H., Poschlod, P., Reich, P.B., Sandel, B., Schamp, B., Sheremetev, S. & Weiher, E. (2018) Plant functional trait change across a warming tundra biome. *Nature*, 562, 57-62. https://doi.org/10.1038/s41586-018-0563-7

Bråthen, K.A., Ravolainen, V.T., Stien, A., Tveraa, T. & Ims, R.A. (2017) Rangifer management controls a climate-sensitive tundra state transition. *Ecol. Appl.* 27, 2416-2427. URL: http://www.jstor.org/stable/26600101

Bryant, J.P., Joly, K., Chapin, F.S., DeAngelis, D.L. & Kielland, K. (2014) Can antibrowsing defense regulate the spread of woody vegetation in arctic tundra? *Ecography*, 37, 204–211. https://doi.org/10.1111/j.1600-0587.2013.00436.x

Bryant, J.P., Provenza, F.D., Pastor, J., Reichardt, P.B., Clausen, T.P. & du Toit, J.T. (1991) Interactions between woody plants and browsing mammals mediated by secondary metabolites. *Ann. Rev. Ecol. Syst.*, 22, 431–446. https://doi.org/10.1146/annurev.es.22.110191.002243

Bryant, J.P., Tahvanainen, J., Sulkinoja, M., Julkunen-Tiitto, R., Reichardt, P. & Green, T. (1989) Biogeographic evidence for the evolution of chemical defense by boreal birch and willow against mammalian browsing. *Am. Nat.*, 134, 20-34. https://doi.org/10.1086/284963

Chapin, F.S., Shaver, G.R., Giblin, A.E., Nadelhoffer, K.J. & Laundre, J.A. (1995) Responses of Arctic tundra to experimental and observed changes in climate. *Ecology*, 76, 694–711. https://doi.org/10.2307/1939337

Christie, K.S., Bryant, J.P., Gough, L., Ravolainen, V.T., Ruess, R.W. & Tape, K.D. (2015) The Role of Vertebrate Herbivores in Regulating Shrub Expansion in the Arctic: A Synthesis. *BioScience*, 65, 1123–1133. https://doi.org/10.1093/biosci/biv137

Crête, M. & Doucet, G.J. (1998) Persistent suppression in dwarf birch after release from heavy summer browsing by caribou. *Arct. Alp. Res.*, 30, 126–132. https://doi.org/10.2307/1552127

Dahlgren, J., Oksanen, L., Oksanen, T., Olofsson, J., Hambäck, P.A. & Lindgren, Å. (2009) Plant defences to no avail? Responses of plants of varying edibility to food web manipulations in a low arctic scrubland. *Evol. Ecol. Res.*, 11, 1189–1203.

ESRI (2011) ArcGIS Desktop: Release 10. Redlands, CA: Environmental Systems Research Institute. *Redlands*.

Flora of North America @ eFloras (2008). Published on the Internet http://www.efloras.org/florataxon.aspx?flora_id=1&taxon_id=233500254, [accessed August 2021]. Missouri Botanical Garden, St. Louis, MO & Harvard University Herbaria, Cambridge, MA.

Gershenzon, J., Fontana, A., Burow, M., Wittstock, U. & Degenhardt, J. (2012) Mixtures of plant secondary metabolites: metabolic origins and ecological benefits. The Ecology of Plant Secondary Metabolites: From Genes to Global Processes. Ecological Reviews, pp. 56-77. Cambridge: Cambridge University Press. https://doi.org/10.1017/CBO9780511675751.005

Goel, G., Puniya, A.K., Aguilar, C.N. & Singh, K. (2005) Interaction of gut microflora with tannins in feeds. *Naturwissenschaften*, 92, 497–503. https://doi.org/10.1007/s00114-005-0040-7

Gowda J.H., Palo R.T. & Udén P. (2019) Seasonal variation in the nutritional value of woody plants along a natural gradient in Eastern Africa. *Afr. J. Ecol.,* 57, 226–237. https://doi.org/10.1111/aje.12583

Graglia, E., Julkunen-Tiitto, R., Shaver, G.R., Schmidt, I.K., Jonasson, S. & Michelsen, A. (2001) Environmental control and intersite variations of phenolics in Betula nana in tundra ecosystems. *New Phytol.*, 151, 227–236. https://doi.org/10.1046/j.1469-8137.2001.00149.x

Gullberg, J., Jonsson, P., Nordström, A., Sjöström, M. & Moritz, T. (2004) Design of experiments: An efficient strategy to identify factors influencing extraction and derivatization of Arabidopsis thaliana samples in metabolomic studies with gas chromatography/mass spectrometry. *Anal. Biochem.*, 331, 283–295. https://doi.org/10.1016/j.ab.2004.04.037

Hartley, I.P., Garnett, M.H., Sommerkorn, M., Hopkins, D.W., Fletcher, B.J., Sloan, V.L., Phoenix, G.K. & Wookey, P.A. (2012) A potential loss of carbon associated with greater plant growth in the European Arctic. *Nature Clim. Change*, 2, 875-879. https://doi.org/10.1038/nclimate1575

Hijmans, R.J., Cameron, S.E., Parra, J.L., Jones, P.G. & Jarvis, A. (2005) Very high resolution interpolated climate surfaces for global land areas. *Int. J. Climatol.*, 25, 1965-1978. https://doi.org/10.1002/joc.1276

Hofgaard, A., Løkken, J.O., Dalen, L. & Hytteborn, H. (2010) Comparing warming and grazing effects on birch growth in an alpine environment - a 10-year experiment. *Plant Ecol. Divers.*, 3, 19–27. https://doi.org/10.1080/17550871003717016

Jayanegara, A. & Palupi, E. (2010) Condensed tannin effects on nitrogen digestion in ruminants: A meta-analysis from in Vitro and in Vivo studies. *Media Peternakan*, 33, 176–181. http://dx.doi.org/10.5398/medpet.2010.33.3.176

Jepsen, J.U., Hagen, S.B., Ims, R.A. & Yoccoz, N.G. (2008) Climate change and outbreaks of the geometrids Operophtera brumata and Epirrita autumnata in subarctic birch forest: evidence of a recent outbreak range expansion. *J. Anim. Ecol.*, 77, 257–264. https://doi.org/10.1111/j.1365-2656.2007.01339.x

Julkunen-Tiitto, R., Rousi, M., Bryant, R.J., Sorsa, S., Keinänen, M. & Sikanen, H. (1996) Chemical diversity of several Betulaceae species: comparison of phenolics and terpenoids in northern birch stems. *Trees*. 11, 16–22. https://doi.org/10.1007/s004680050053

Krizsan, S.J., Mateos-Rivera, A., Bertilsson, S., Felton, A., Ramin, M., Vaga, M., Gidlund, H., Huhtanen, P. (2018) An in vitro evaluation of browser and grazer fermentation efficiency and microbiota using European moose spring and summer foods. *Ecol. Evol.* 8,4183-4196. https://doi.org/10.1002/ece3.3920

Kramer, P.J. & Kozlowski, T.T. (1979) Physiology of Woody Plants, Academic Press, New York.

Kumar, S. & Pandey, A.K. (2013) Chemistry and biological activities of flavonoids: An overview. *Sci. World. J.*, vol 2013, article ID 162750. https://doi.org/10.1155/2013/162750

Lindgren, E. (1979). Vallfodrets näringsvärde bestämt in vivo och med olika laboratoriemetoder (The nutritional value of roughages determined in vivo and by laboratory methods). Swedish University of Agricultural Science, Department of animal husbandry, Report 45, Uppsala, Sweden.

Manseau, M., Huot, J. & Crete, M. (1996) Effects of Summer Grazing by Caribou on Composition and Productivity of Vegetation: Community and Landscape Level. *J. Ecol.*, 84, 503-513. URL: https://www.jstor.org/stable/2261473

Marsch, K.J., Wallis, I.R, Kulheim, C., Clark, R., Nicolle, D. Foley, W.J. & Salminen, J-P. (2020) New approaches to tannin analysis of leaves can be used to explain in vitro biological activities associated with herbivore defence. *New Phytol.*, 225, 488-498. https://doi.org/10.1111/nph.16117

McLean, S., Richards, S.M., Cover, S.L., Brandon, S., Davies, N.W., Bryant, J.P. & Clausen, T.P. (2009) Papyriferic acid, an antifeedant triterpene from birch trees, inhibits succinate dehydrogenase from liver mitochondria. *J. Chem. Ecol.*, 35, 1252-1261. https://doi.org/10.1007/s10886-009-9702-9

Minchin, P.R. (1987) An evaluation of the relative robustness of techniques for ecological ordination. *Vegetatio*, 69, 89-107. https://doi.org/10.1007/BF00038690

Myers-Smith, I.H., Forbes, B.C., Wilmking, M., Hallinger, M., Lantz, T., Blok, D., Tape, K.D., MacIas-Fauria, M., Sass-Klaassen, U., Lévesque, E., Boudreau, S., Ropars, P., Hermanutz, L., Trant, A., Collier, L.S., Weijers, S., Rozema, J., Rayback, S.A., Schmidt, N.M., Schaepman-Strub, G., Wipf, S., Rixen, C., Ménard, C.B., Venn, S., Goetz, S., Andreu-Hayles, L., Elmendorf, S., Ravolainen, V., Welker, J., Grogan, P., Epstein, H.E. & Hik, D.S. (2011) Shrub expansion in tundra ecosystems: Dynamics, impacts and research priorities. *Environ. Res. Lett.,* 6, 045509. http://dx.doi.org/10.1088/1748-9326/6/4/045509

Oksanen, J., Blanchet, F.G., Friendly, M., Kindt, R., Legendre, P., Mcglinn, D., Minchin, P.R., O'hara, R.B., Simpson, G.L., Solymos, P., Henry, M., Stevens, H., Szoecs, E. & Maintainer, H.W. (2019) vegan: Community Ecology Package. R package version 2.5-5. https://CRAN.R-project.org/package=vegan

Olofsson, J., Oksanen, L., Callaghan, T., Hulme, P.E., Oksanen, T. & Suominen, O. (2009) Herbivores inhibit climate-driven shrub expansion on the tundra. *Glob. Chang. Biol.*, 15, 2681–2693. https://doi.org/10.1111/j.1365-2486.2009.01935.x

Pichersky, E. & Raguso, R.A. (2018) Why do plants produce so many terpenoid compounds? *New Phytol.*, 220, 692–702. https://doi.org/10.1111/nph.14178

Porter, L.J., Hrstich, L.N. & Chan, B.G. (1985) The conversion of procyanidins and prodelphinidins to cyanidin and delphinidin. *Phytochemistry*, 25, 223–230. https://doi.org/10.1016/S0031-9422(00)94533-3

Post, E. & Pedersen, C. (2008) Opposing plant community responses to warming with and without herbivores. *Proc. Natl. Acad. Sci. U.S.A.*, 105, 12353–12358. https://doi.org/10.1073/pnas.0802421105

Prendin, A.L., Carrer, M., Karami, M., Hollesen, J., Pedersen, N.B., Pividori, M., Treier, U.A., Westergaard-Nielsen, A., Elberling, B. & Normand, S. (2019) Immediate and carry-over effects of insect outbreaks on vegetation growth in West Greenland assessed from cells to satellite. *J. Biogeogr.*, 47, 87-100. https://doi.org/10.5061/dryad.36kj534

Price, M.L., Scoyoc, S. van & Butler, L.G. (1978) A critical evaluation of the vanillin reaction as an assay for tannin in Sorghum grain. *J. Agric. Food. Chem.*, 26, 1214–1218. https://doi.org/10.1021/jf60219a031

R Core Team (2020). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL: https://www.R-project.org/

Reichardt, P.B., Bryant, J.P., Clausen, T.P. & Wieland, G.D. (1984) Defense of winter-dormant Alaska paper birch against snowshoe hares. *Oecologia*, 65, 58-69. URL: https://www.jstor.org/stable/4217494

Salminen, J-P., Ossipov, V., Pihlaja, K. (2002) Distribution of hydrolysable tannins in the foliage of Finnish birch species. *Z. Naturforsch. C.*, 57, 248-256. https://doi.org/10.1515/znc-2002-3-409

Salminen, J-P. & Karonen, M. (2011) Chemical ecology of tannins and other phenolics: we need a change in approach. *Funct. Ecol.,* 25, 325-338. https://doi.org/10.1111/j.1365-2435.2010.01826.x

Schneider, C.A., Rasband, W.S. & Eliceiri, K.W. (2012) NIH Image to ImageJ: 25 years of image analysis. *Nature Methods*, 9, 671-675. https://doi.org/10.1038/nmeth.2089

Skarin, A., Verdonen, M., Kumpula, T., Macias-Fauria, M., Alam, M., Kerby, J., Forbes, B.C. (2020) Reindeer use of low Arctic tundra correlates with landscape structure. *Environ. Res. Lett.*, 15, 115012. http://doi.org/10.1088/1748-9326/abbf15

Sturm, M. (2005) Changing snow and shrub conditions affect albedo with global implications. *J. Geophys. Res.*, 110, G1. https://doi.org/10.1029/2005JG000013

Torp, M., Witzell, J., Baxter, R. & Olofsson, J. (2010) The effect of snow on plant chemistry and invertebrate herbivory: Experimental manipulations along a natural snow gradient. *Ecosystems*, 13, 741–751. https://doi.org/10.1007/s10021-010-9351-4

Tremblay, B., Lévesque, E. & Boudreau, S. (2012) Recent expansion of erect shrubs in the Low Arctic: Evidence from Eastern Nunavik. *Environ. Res. Lett.*, 7, 035501. http://dx.doi.org/10.1088/1748-9326/7/3/035501

White, R.G. & Lawler, J.P. (2002) Can methane suppression during digestion of woody and leafy browse compensate for energy costs of detoxification of plant secondary compounds? A test with muskoxen fed willows and birch. *Comp. Biochem. Physiol. A Mol. Integr. Physiol.*, 133, 849-59. https://doi.org/10.1016/s1095-6433(02)00152-6

Wright, I.J., Reich, P.B., Westoby, M., Ackerly, D.D., Baruch, Z., Bongers, F., Cavender-Bares, J., Chapin, T., Cornelissen, J.H., Diemer, M., Flexas, J., Garnier, E., Groom, P.K., Gulias, J., Hikosaka, K., Lamont, B.B., Lee, T., Lee, W., Lusk, C., Midgley, J.J., Navas, M.L., Niinemets, U., Oleksyn, J., Osada, N., Poorter, H., Poot, P., Prior, L., Pyankov, V.I., Roumet, C., Thomas, S.C., Tjoelker, M.G., Veneklaas, E.J. & Villar, R. (2004) The worldwide leaf economics spectrum. *Nature*, 428, 821-827. https://doi.org/10.1038/nature02403