Measuring Background Levels of Invertebrate Herbivory in the Arctic Tundra

by

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Abstract

Climate change will continue to affect the Arctic more intensely than other biomes. These changes can have dramatic effects on biotic interactions that influence the functioning of these systems, including plant-herbivore interactions. Invertebrate herbivores strongly depend on external temperatures for their growth and metabolism and as temperatures in tundra ecosystems increase, increases in the rates of invertebrate herbivory are expected. However, little is known about the current levels of invertebrate herbivory in tundra, and critical information is missing in order to evaluate future changes. This project set out to determine what the baseline level of invertebrate herbivory is, how it varies, and what factors are driving variation across the tundra biome. Utilizing a standardized protocol designed by *The Herbivory Network,* data was collected from 22 tundra sites during summer 2015. This protocol outlined the methods for leaf collection, so that all data collection was consistent. Data was collected at both the species and community level, as well as at two different spatial scales; sites (0.25-25 km^2), and plots within the sites (20x20 m). Leaves were collected from the "focal species", defined as the three most common plants species at each plot. Leaves were analysed individually to determine the type of herbivory and the area lost to invertebrate herbivores. At the species level, a total of 45 different focal species were collected. Of those species, four were selected as "target species" occurring in ≥6 sites, thus allowing for a comparison across sites; *Betula nana, Vaccinium vitis-idaea, Empetrum nigrum* and *Salix polaris/herbacea*. The drivers of invertebrate herbivory were species-specific. *E. nigrum* showed minimal variation due to its low palatability. Climatic variables such as temperature and precipitation explained some of the variation in herbivory for *B. nana* and *S. polaris/herbacea* (temperature only). Latitude and collection date were found to partially drive the variation in *B. nana* and *V. vitis-idaea*, although in different directions. Increasing latitude had a positive effect on *B. nana* herbivory and later collection date had a negative effect, while *V. vitis-idaea* showed the opposite. Most of the variation in leaf damage was found within sites, between the different plots, rather than between the individual samples within a plot, suggesting that local characteristics play an important role in determining species level herbivory. At the community level none of the included predictor variables (temperature, precipitation, and collection date) were found to drive the variation in herbivory. Similar for the species level herbivory, most of the variation was driven by unidentified local (within site) characteristics. Overall, invertebrate herbivory was prevalent across the tundra biome, occurred at low intensity, and varied between sites. Both temperature and, in some cases, precipitation were associated with increased levels of herbivory on some of the focal species, and this could result in increased herbivory as climate continues to warm. Climatic variables did not explain the variation in herbivory at the community level, but this could be due to climate having a stronger species-specific effect that was masked by combining species. Further work is required to determine the specific drivers of invertebrate herbivory in order to make more accurate predictions about these impacts on tundra ecosystems in the future.

Preface

This thesis was a collaborative work done through *The Herbivory Network*. Data was collected following a common protocol (**Appendix 1**) designed by Dr. Isabel Barrio and Dr. Mikhail Kozlov in 2015. Many different researchers contributed to data collection (**Table 2.1**). The sampling method was designed and data was collected prior to my involvement in this project. As this is a collaborative project, I attended meetings with *The Herbivory Network* in Turku, Finland in February 2016, and in Reykjavík, Iceland in September 2016, where I presented updates on the progress of the project.

Acknowledgments

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List of Abbreviations

PLD → Percent leaves damaged

PLAD → Percent leaf area damaged

APAD → Average percent leaf area damaged

CWB → Community weighted biomass lost to invertebrate herbivory

CAVM → Circumpolar Arctic Vegetation Map

LMM → Linear Mixed Effects Model

VIF → Variance Inflation Factor

LRT → Log-Likelihood Ratio test

CF → Conversion Factor

GDD → Growing degree days

Plant species abbreviations

A. alpina → *Arctous alpina* (L.) Nied

B. nana → *Betula nana* (L.)

C. arcticum → *Cerastium arcticum* Lange

D. octopetala → *Dryas octopetala* L.

E. nigrum → *Empetrum nigrum* L.

L. spicata → *Luzula spicata* (L.) DC.

S. arctica → *Salix arctica* Pall.

S. herbacea → *Salix herbacea* L.

S. polaris → *Salix polaris* Wahlenb.

S. reticulata → *Salix reticulata* L.

V. myrtillus → *Vaccinium myrtillus* L.

V. uliginosum → *Vaccinium uliginosum* L.

V. vitis-idaea → *Vaccinium vitis-idaea* L.

Chapter 1: Background invertebrate herbivory

The relationship between plants and herbivores is critical to the overall functioning of an ecosystem (Burkepile and Parker 2017). Patterns of invertebrate herbivory and their resulting effects on plants have been examined mainly in the tropics (Marquis 1984, Coley and Barone 1996) and in monetarily valuable ecosystems such as crops and forests (Stephens 1971, Cranshaw and Radcliffe 1978, Stewart et al. 1990). However, these same patterns have not been as extensively studied in tundra ecosystems (but see Kozlov et al. 2015b, Barrio et al. 2017). The few studies investigating patterns of invertebrate herbivory in tundra ecosystems have looked at a single host plant species (Barrio et al. 2017) or at specific growth forms (shrubs, Kozlov et al. 2015b), but to-date none has assessed the patterns of invertebrate herbivory at the community level. The intensity and spatial distribution of invertebrate herbivory can determine the effects that herbivores have on plant growth and reproduction, nutrient cycling, and plant community composition. Investigating patterns of invertebrate herbivory at the community level and how these are related to environmental drivers is particularly relevant for tundra ecosystems that are rapidly changing.

In this thesis, I investigate the patterns of leaf damage by invertebrate herbivores across the tundra biome, estimate biomass loss at the species and community level for dominant tundra habitat types, and how these patterns relate to main environmental drivers (latitude and climate). To ensure comparability of results across different tundra sites a common protocol was used (**Appendix 1**).

Invertebrate herbivory in tundra

Herbivory by invertebrates in tundra ecosystems has been generally assumed to be negligible (Haukioja 1981, MacLean Jr. 1981)**.** These early conclusions were probably due to the lower diversity and abundance of invertebrate herbivores found in the tundra compared to other ecosystems (Haukioja 1981, Hodkinson et al. 2013). The proportion of herbivorous taxa, relative to that of other terrestrial and aquatic invertebrates strongly decreases from temperate areas towards the High Arctic (Danks 1992). As well, the impact of invertebrate herbivores appears to be low due to the removal of relatively small amounts of biomass, compared to the larger amounts consumed by vertebrate herbivores (Kotanen and Rosenthal 2000).

However, some species of invertebrate herbivores can undergo outbreaks, during which they consume massive amounts of plant biomass over a short time period. Outbreaks have been mostly reported for boreal forests and the forest-tundra ecotone (Zvereva et al. 2012, Kozlov and Zvereva 2014, Metcalfe et al. 2016), and few have been described in true tundra systems (Post and Pedersen 2008, Lund et al. 2017). Outbreaks have been shown to cause the death of many plant individuals (Jepsen et al. 2008), alter nutrient cycling and belowground community composition (Kaukonen et al. 2013), and lead to shifts in the composition of dominant plant species (Jepsen et al. 2013). In contrast, under non-outbreak densities, invertebrates cause small chronic biomass removal, this is referred to as background herbivory (Zvereva et al. 2012, Kozlov et al. 2015b). With the current focus mainly on outbreaks there are only a few studies that expand our knowledge on the patterns of background herbivory (Kozlov et al. 2015a).

At background densities, immediate effects of invertebrates appear minimal (Kotanen and Rosenthal 2000), but the longer-term nature of background herbivory may lead to lasting effects on plant growth and reproduction (Zvereva et al. 2012), community interactions (Barrio et al. 2013), and influence nutrient fluxes (Metcalfe et al. 2016). In boreal forest ecosystems an annual foliar biomass loss of 2-15% can be attributed to background invertebrate herbivory (Zvereva et al. 2012), while in tundra systems background invertebrate herbivory removes <1% of the foliar biomass (Kozlov et al. 2015a). In their study on mountain birch (*Betula pubescens* subsp. *czerepanovii*), Zvereva et al. (2012) observed that the effects of small continuous biomass loss (5-18%) over multiple years resulted in a decrease in plant growth. A similar decrease was observed in plants that had had 100% of their leaf biomass removed during an outbreak in a single year. These results demonstrate that the constant stress of minor herbivory can considerably affect plant growth, and potentially the evolution of herbivory defenses (Kozlov et al. 2015b) and tolerance (Rosenthal and Kotanen 1994).

The interaction between invertebrate herbivores and plants in tundra ecosystems exist under particular environmental conditions (Strathdee and Bale 1998). The tundra biome is characterized by cold temperatures, a short growing season, precipitation that falls mostly as snow (ranging from dry polar deserts to waterlogged soil), low nutrient soil that is often frozen or mostly frozen, and slow decomposition rates (Strathdee and Bale 1998, Mulder 1999). The biome can be broken down into three sub-biomes that have more definite descriptions: arctic tundra, ericoid-graminoid tundra, and oroarctic or alpine tundra (Virtanen et al. 2016). The arctic tundra is defined as being very cold and dry (polar desert), with soils that are frozen for most of the year. Ericoid-graminoid tundra has more snow and slightly warmer temperatures with greater plant cover. The alpine tundra, or oroarctic sub-biome, is defined as mid- and low- latitude mountain tundra with lightly frozen soils and graminoid dominated vegetation (Virtanen et al. 2016).

Climate change is expected to have the strongest effects in tundra ecosystems (Post et al. 2009), The Arctic tundra has already faced an increase of 2-3°C in the average temperature since the 1950's, as well as increases in the amount of precipitation (Huntington et al. 2005, Overland et al. 2017). Invertebrates are ectotherms and therefore rely on external temperatures for metabolism and life history (Danks 2004). Climate change in the tundra is therefore likely to cause many changes, directly and indirectly (through plant phenology shifts), to the life cycles and behaviour of many invertebrate species (Bale et al. 2002). For example, increased winter temperatures can affect the overwinter survivorship of some species of invertebrates, leading to more individuals emerging and resulting in outbreaks of feeding stages (Wallner 1987, Strathdee and Bale 1998, Bale et al. 2002). As temperatures continue to increase, the occurrence of these outbreaks is likely to become more frequent (Bale et al. 2002, Kozlov 2008). Warmer weather also facilitates the expansion of invertebrate herbivores into habitats that were previously too cold and un-exposed to outbreaks (Wolf et al. 2008). While warmer winters appear to determine outbreak dynamics, changes in summer temperatures appear to alter background herbivory (Kozlov 2008). The main growth phase of most northern invertebrates occurs during the summer; as a result, higher summer temperatures can accelerate growth and development in many species (Bale et al. 2002). This would generally result in increased background herbivory without outbreak cycles, and lead to greater impacts than outbreaks in the long term (Wolf et al. 2008). With the potential for so many different responses to climate change it is essential to understand the existing patterns of invertebrate herbivory in order to assess future responses.

Why study patterns of invertebrate herbivory?

Patterns in the distribution, extent, and duration of invertebrate herbivory will ultimately determine what effect it will have on plant growth and reproduction, community structure, and soil nutrients. Most studies on the effects of invertebrate herbivory on plants refer to biomes other than tundra (but see Jepsen et al. 2008, Barrio et al. 2017), so many of the effects of invertebrate herbivory on tundra plants have to be inferred from studies conducted at more southerly latitudes.

Invertebrate herbivores consume different plant organs, from leaves, flowers or seeds to roots, bark or phloem fluids. Leaf herbivory is the most apparent type of damage by invertebrate herbivores. Defoliation by invertebrates directly depletes the amount of photosynthetic tissue available, thus affecting plant growth and reproduction (Franklin 1970, Harris 1974, Kozlov and Zvereva 2014). Leaf consumption by invertebrates also has indirect effects on the plant community such as altering the nutrient input into the soil (Hunter 2001, Kaukonen et al. 2013, Metcalfe et al. 2016) and changing plant community composition (Mulder et al. 1999, Wolf et al. 2008, Jepsen et al. 2013, Karlsen et al. 2013).

In many plants there is a damage threshold below which the plant exhibits no visible negative effect on growth or reproduction (Hendrix 1988, Strauss and Agrawal 1999). Plants generally tolerate a single small loss of leaf tissue to herbivores (Stephens 1971, Zvereva et al. 2012), but after damage accumulates or repeated attacks occur these negative effects can be observed (Rafes 1970, Jefferies et al. 1994, Kotanen and Rosenthal 2000, Zvereva et al. 2012).

The accumulated leaf biomass lost to herbivory results in a heavy reliance on the plant's carbohydrate stores due to reduced photosynthetic ability (Maun and Cavers 1971, Crawley 1983). Plants can respond to defoliation by invertebrate herbivores by shifting resources either away from growth or into regrowth (Crawley 1983). Decreases in growth occur when large amounts of leaf area is lost and the plant needs to allocate resources directly to survival (Rafes 1970, Marquis 1984). Alternatively, when small amounts of damage occur, plants can undergo regrowth in order to compensate for lost leaf tissue (Islam and Crawley 1983, Trumble et al. 1993, Rosenthal and Kotanen 1994, Mulder 1999, Ohgushi 2008).

The effects of invertebrate herbivory on plant reproduction can be diverse. Invertebrate leaf herbivory can result in delayed flowering time (Marquis 1984) and flower maturation (Islam and Crawley 1983). Both of these delays can lead to pollinator mismatches, or the delay can be so late that frost may damage flowers before they are able to fruit (Islam and Crawley 1983, Marquis 1984). Defoliation by invertebrate herbivores can also reduce seed production (Marquis 1984, Lehtilä and Strauss 1999), decrease the size of seeds (Maun and Cavers 1971), or limit seed viability (Marquis 1984).

In some cases, repeated defoliation can lead to plant mortality. Mortality of an individual often only occurs if damage is repeated over time (Stephens 1971), or when extreme defoliation (>75%) events occur (Reeks and Barter 1951, Hendrix 1988). Most studies on plant mortality and defoliation have focused on non-clonal plant species, and the effects on clonal species (such as many tundra plants) have not been extensively examined. As a secondary effect, invertebrate herbivory can open the door to plant diseases or fungi that subsequently lead to plant death (Hendrix 1988, Olofsson et al. 2013).

Invertebrate leaf herbivory can also affect nutrient cycling. Leaf litter is a main vector for the cycling of nutrients, such as nitrogen and phosphorous, back into the soil from plants. When leaves fall naturally they first undergo senescence, during which up to 70% of the nitrogen in the leaf tissue is resorbed by the plant (Hunter 2001, Metcalfe et al. 2016). This results in natural leaf litter being low in nutrients and difficult to decompose, especially in the sub-arctic and arctic soils where decomposition is already a slow process (Kaukonen et al. 2013). This flux of nutrients between plants and soil can be drastically altered by invertebrate herbivory. During outbreaks invertebrates contribute massive amounts of frass (excrement), molts, and cadavers directly to the soil (Hunter 2001, Kaukonen et al. 2013, Metcalfe et al. 2016). Invertebrate herbivores can also cause premature leaf abscission (Faeth et al. 1981, Zvereva and Kozlov 2014); these leaves, along with pieces of chewed leaves, reach the soil without undergoing complete senescence and therefore have higher levels of nitrogen (Hunter 2001). The increase in soil nutrients can result in more availability of nitrogen for plants (Lund et al. 2017); this was observed by Post and Pedersen (2008) when they found leaves had four times more nitrogen during an outbreak than before an outbreak. While outbreaks represent the extreme for nutrient inputs by invertebrate herbivores, background levels of herbivory still result in a steady input of frass, molts, and cadavers that lead to increased soil nutrients (Hunter 2001, Metcalfe et al. 2016).

Defoliation by invertebrate herbivores can also lead to changes in the decomposer community structure by altering the soil nutrient levels and the soil microclimate (Kaukonen et al. 2013). Invertebrate herbivores change the soil microclimate by altering canopy coverage, thus allowing more sunlight and precipitation to reach the soil (Jepsen et al. 2013, Kaukonen et al. 2013).

Finally, herbivores can induce significant changes in plant community composition. The selective feeding of herbivores on certain plants results in decreased abundance of these species and also leads to shifts in the competitive ability of the plants in that community (Post and Pedersen 2008). For example, shrub expansion into the tundra can be buffered by selective feeding by mammalian herbivores (Olofsson et al. 2004). The same type of shifts can also be linked to invertebrate herbivores, especially during outbreaks. For instance, the expansion of *Betula nana* was shown to be halted by a combination of environmental factors and invertebrate herbivory (Gamm et al. 2017). The extreme foliage loss during outbreaks decreases the biomass of the preferred food plant species and potentially decreases their competitive ability (Mulder et al. 1999). In sub-arctic birch forests, geometrid moth larvae outbreaks can result in shifts in the understory plant communities, from a woody shrub dominated community to one dominated by graminoids (Jepsen et al. 2013, Karlsen et al. 2013). In this forest, the dominant understory shrubs included *Betula nana* and *Empetrum nigrum*. The outbreak resulted in larvae consuming high levels of *B. nana* once their preferred host plant was depleted. The unpalatable *E. nigrum* also decreased in biomass, probably due to starving larvae attempting to consume some leaves. Other studies have shown that after defoliation *E. nigrum* can become susceptible to subsequent fungal infections (Olofsson et al. 2013). By decreasing the abundance of *B. nana* shrubs and releasing the area from the competitively exclusive *E. nigrum* the outbreak allowed for the expansion of the graminoid *Avenella flexuosa* (Jepsen et al. 2013, Karlsen et al. 2013). Similar effects might be expected during background levels of invertebrate herbivory since the accumulated loss of leaf tissue results in decreased plant performance that can be equal or greater than during outbreaks (Wolf et al. 2008, Zvereva et al. 2012).

Potential drivers of invertebrate herbivory in tundra

Large-scale patterns of the intensity of biotic interactions have been the object of study of biogeographers and macroecologists for decades. Most hypotheses proposed to explain these patterns include latitude or the effect of climate variables. In this study I will focus on three different potential drivers of the patterns of invertebrate herbivory in tundra: latitude, temperature, and precipitation.

The Latitudinal Herbivory Hypothesis indicates that there is a linear decrease in herbivory as you increase in latitude from the Equator, such that temperate forests have less herbivory than tropical forests (Coley and Barone 1996). This hypothesis defined herbivory such that it included invertebrate and mammalian herbivores together. A more detailed picture, specific to invertebrate herbivory, was determined by Kozlov et al. (2015b), when they examined the Latitudinal Herbivory Hypothesis including polar regions. They found that while polar areas had lower levels of invertebrate herbivory than tropical and temperate zones, the change in herbivory level was not linear but dome-shaped. Kozlov et al. (2015b) concluded that the latitudinal pattern of herbivory was dependent on the climate zone, such that tropical forests had no latitudinal gradient of herbivory, temperate forests had a slight decrease in herbivory with latitude, and the polar zone had a strong decrease in invertebrate herbivory with increased latitude. These patterns in total leaf biomass lost were attributed to the temperature gradient in each zone. Latitudinal trends in herbivory have also been attributed to lower herbivore diversity at high latitudes (Anstett et al. 2016), changes in plant defensive chemistry (Moles et al. 2011), predator pressure (Björkman et al. 2011) or even variations in leaf toughness (Onoda et al. 2011).

Direct effects of temperature on invertebrate herbivores can vary depending on the life history of the herbivore and the climate zone where the invertebrate is found (Bale et al. 2002, Kozlov et al. 2015b). Invertebrate physiology strongly depends on temperature, therefore moderate increases in temperature have the potential to shift life cycle durations, increase the density of invertebrates, and shift their distributions (Hodkinson and Bird 1998, Bale et al. 2002). When looking at the temperate zone, invertebrate herbivores may have an increase in the number of generations per year for some species, or faster seasonal growth rates for others (Bale et al. 2002). Warmer temperatures may allow many species to expand their ranges into higher latitudes and altitudes, while some cold-adapted species are expected to shift their southern range north to avoid warmer temperatures (Bale et al. 2002, Jepsen et al. 2008). Higher temperatures can also be linked to greater winter survival (Callaghan et al. 2004, Wolf et al. 2008), higher herbivore density (Bale et al. 2002), and altered feeding activity (Birkemoe et al. 2016). Due to the effect temperature has on invertebrate herbivores it can be predicted that changes in temperature will results in changes in herbivory patterns.

The effect of precipitation on herbivory has focused more on the effects precipitation has on plants rather than on the invertebrates. Decreases in water availability can lead to increased leaf toughness, making them more resistant to herbivore damage (Onoda et al. 2011). Stress due to drought can induce plants to decrease the production of herbivore defense chemicals, resulting in an increase in the palatability of leaf tissue (Berg et al. 2008). In their study on latitudinal patterns of herbivory, Kozlov et al. (2015b) found that the pattern in herbivory for defoliating herbivores was best predicted by precipitation in all three climatic zones (polar, temperate, and tropical) such that increased precipitation resulted in higher levels of defoliation damage.

The drivers of patterns in invertebrate herbivory may also differ depending on the feeding type and/or life history of the herbivore (Hiura and Nakamura 2013, Moreira et al. 2015). In this study, I examine patterns of leaf damage caused by externally feeding herbivores (chewing and skeletonizing), and two types of damage caused by internally feeding herbivores: mining damage (serpentine and window) and gall damage. External feeding marks can involve the consumption of all layers of leaf tissue (i.e. chewing damage, **Figure 1.1A**), or the consumption of only the external epidermal layers (i.e. skeletonization, **Figure 1.1B**). External damage has been identified as the main contributor to leaf damage in tundra, compared to mining and galling (Kozlov et al. 2015b, Barrio et al. 2017). The low incidence of leaf damage by miners and gallers has been a consistent trend through the fossil record as well (Currano et al. 2008). Leaf mines, in the form of serpentine mines (**Figure 1.1C**) or window mines (**Figure 1.1D**) are caused by invertebrate herbivores feeding internally between leaf epidermises. Leaf galls are caused by an invertebrate herbivore inducing the plant to create abnormal growth of plant tissues (**Figure 1.1E, F**). Invertebrates that feed externally are more exposed to environmental conditions and may respond to them directly, whereas those feeding from the inside of the leaves (leaf gallers and miners) are more protected by leaf tissues and may be less affected by abiotic variables. For example, Mosbacher et al. (2013) found that the patterns in gall herbivory were not as affected by climatic effects as by the ability for gall mites to disperse.

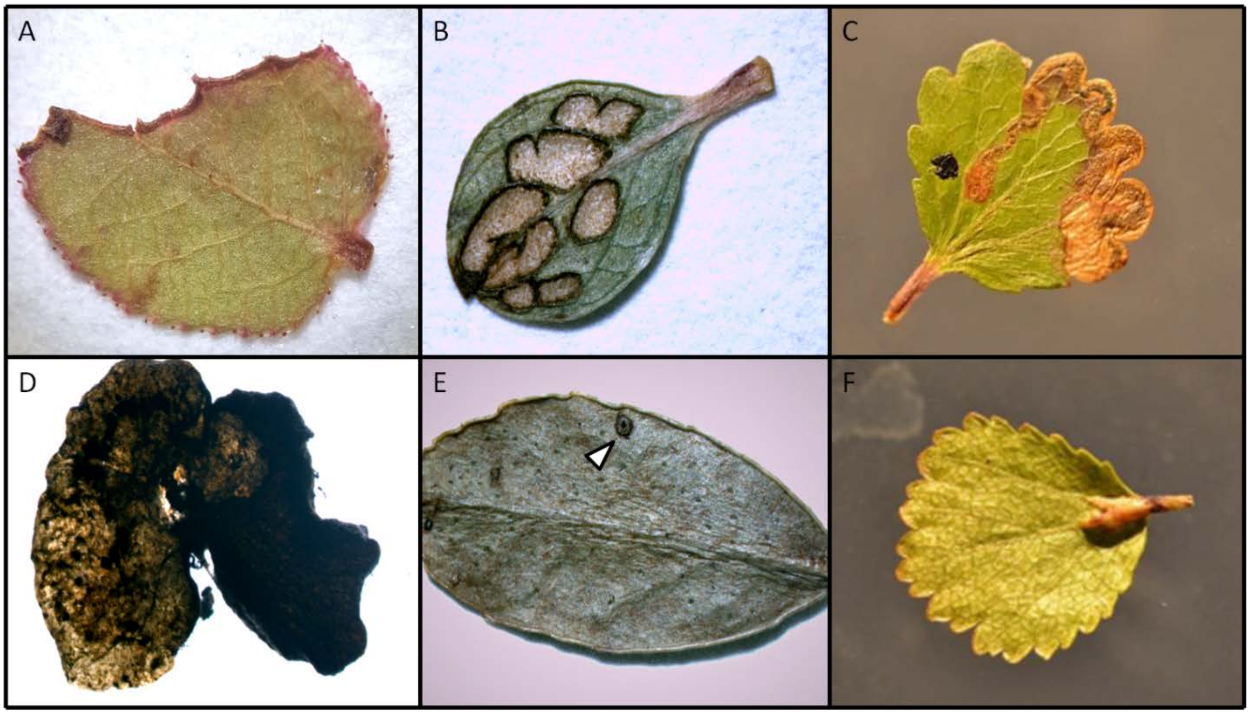

Figure 1.1 Photographs of different types of leaf damage caused by invertebrate herbivores on tundra plants. A: Chewing damage on *Vaccinium myrtillus.* B: Skeletonization damage on *Salix rotundifolia*. C: Serpentine mining damage in *Betula nana.* D: Window mining damage in *Oxyria digyna*. E: Gall damage on *Vaccinium vitis-idaea* (white arrow head indicates where the gall is located). F: Petiole gall damage on *Betula nana*.

The need for a common protocol

In order to assess the variation in patterns of invertebrate herbivory in the tundra, different habitat types need to be examined across the whole biome. Comparable measures can be collected across habitat types using a carefully designed common protocol. Common protocols outline a standardized methodology that would be consistently applied to different study sites. Samples would be collected in the same fashion by multiple researchers, thus allowing for adequate comparisons between different sites (Fraser et al. 2013). Common protocols for data collection have been suggested for ecological studies since many of the current questions regarding climate change are global issues that extend over different ecosystems (Henry and Molau 1997, Fraser et al. 2013, Sternberg and Yakir 2015). Common protocols can be effectively applied over large scales to address general ecological questions. According to Anstett et al. (2016) the use of common protocols could help clarify the mixed results regarding latitudinal patterns in herbivory. By implementing a consistent form of measurement a comprehensive comparison of herbivore damage at different latitudes can be done regardless of the biome (temperate vs. tropical vs. polar). Similarly, common protocols can help collect comparable measurements across heterogeneous ecosystems. For example, tundra ecosystems can be highly variable in relation to habitat type, climate, plant phenology, and the species composition of both plants and invertebrates (Danks 1981); therefore common protocols should be developed to investigate plant-herbivore interactions in a consistent way across tundra ecosystems. Standardized protocols have already been successfully applied to arctic and alpine tundra ecosystems to examine the effect of warming on plant communities (International Tundra Experiment, ITEX; Henry and Molau 1997).

A main advantage of common protocols is that they can be implemented by many different researchers at different sites. However, this can also become a disadvantage and the potential issue of observer differences needs to be controlled for. In the case of common protocols aimed at assessing invertebrate herbivory, special attention needs to be placed on assessing the extent of herbivore damage in a consistent way between different observers. Previous studies have found that, while visual estimates can be cheap and accurate, there is still some potential variation in estimation due to observer effects (Johnson et al. 2016). Part of this study (**Appendix 2**) will examine how much variation can be caused by different observers assessing the same samples in order to determine whether multiple observers can be utilized in data analysis for this protocol. The results of this study will help answer some questions that will lead towards the development of a common protocol for measuring invertebrate herbivory.

Conclusion

Invertebrate herbivory can have strong influences on plant growth and reproduction (Maun and Cavers 1971, Rosenthal and Kotanen 1994, Zvereva et al. 2012), nutrient flux (Hunter 2001, Kaukonen et al. 2013), and the composition of plant communities (Karlsen et al. 2013). Many of these effects have been examined in ecosystems other than tundra, so the effects of invertebrate herbivory in the tundra need to be inferred from results from other ecosystems, which may not necessarily hold. The effects of invertebrate herbivory will depend on what the patterns of invertebrate herbivory are. Potential drivers of these patterns include latitude and climate (temperature and precipitation) (Kozlov et al. 2015b). This study aims to identify what the patterns of leaf damage by invertebrate herbivores across the tundra biome are, and how they relate to the main environmental drivers. The outcomes will allow for a more comprehensive understanding of the variation in invertebrate herbivory, and utilize this knowledge to formulate a common protocol to assess future changes in these patterns.

Chapter 2: Description of Study Sites and Focal Species

Introduction

Data collection for this study was conducted in summer 2015 across the circumpolar region, and included a number of lower latitude alpine sites. Researchers contributed data from a total of 22 arctic/alpine tundra study sites, in 9 different countries (**Table 2.1, Figure 2.1**). This design allowed us to incorporate a wide range of variability of tundra ecosystems (**see Chapter 3**). At each site, the dominant habitat type was selected and it was recommended that 5 sampling plots were established (but the number of plots ranged between 1 and 9; **Figure 2.1**). The most abundant plant species in each plot (hereafter 'focal species') were sampled. Thus, the study includes a total of 45 focal species spread across the 22 study sites (**Table 2.2**). The majority of these species only occur as focal species in ≤ 3 sites; to examine the species level variation in invertebrate herbivory only the species that are found in 6 or more sites were considered, and are described here. These species include *Betula nana, Empetrum nigrum, Vaccinium vitis-idaea,* and the combination of *Salix herbacea* and *Salix polaris.* In this Chapter each of the study sites and the main focal species (hereafter 'target species') are described in more detail.

Study Sites

The following site descriptions include the location, details about the habitat type, vegetation type classification following the Circumpolar Arctic Vegetation Map (CAVM 2003), average July temperature and precipitation from 1990-2015, average July temperature for 2015, cumulative July precipitation for 2015, and either the tundra sub-biome as defined by Virtanen et al. (2016) or as defined by CAVM (2003). The study sites are presented alphabetically by country and then site name.

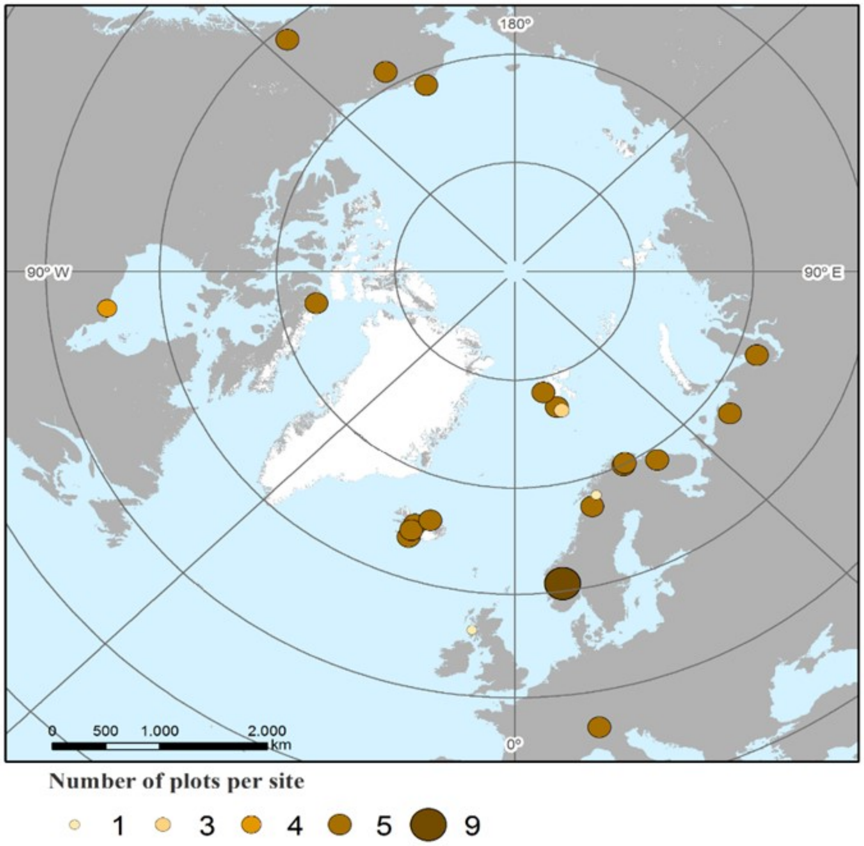

Figure 2.1 Location of the 22 study sites in the Arctic, sub-Arctic, and one alpine site in the Alps. Dot size and colour indicate the number of plots sampled in each site.

CANADA

Burntpoint Creek

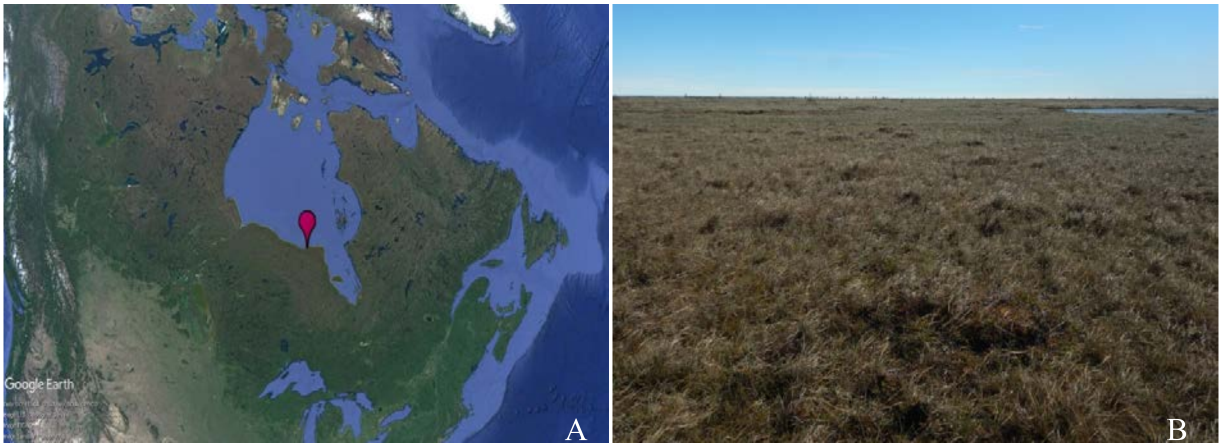

Figure 2.2 Burntpoint Creek located in northern Ontario, Canada (A), with tundra meadow-marsh habitat (B). (Photo credit: Ministry of Natural Resources).

'Burntpoint Creek' is located in northern Ontario, Canada (55.24°N, 84.32°W). Situated approximately 5 km south of the coast of Hudson's Bay, the four plots in this site range in elevation from 7-8 m a.s.l. Burntpoint Creek is in an open meadow-marsh with small areas of standing freshwater. Vegetation cover is 90-95% and is dominated by graminoids with dwarf birch shrubs, willow shrubs, and small herbs/forbs scattered throughout the graminoid community. The vegetation type for this area is classified as wetland (sedge, moss, dwarf shrub wetland, W2; CAVM 2003). The focal plant species for this site, by cover, are *Carex aquatilis, Trichophorum cespitosum,* and *Andromeda polifolia.* Based on the definitions outlined by Virtanen et al. (2016) for the tundra sub-biomes, Burntpoint Creek is classified as arctic tundra**.** Burntpoint Creek has an average July temperature and rainfall (1990-2015) of 14.8°C and 85.3 mm, respectively (Harris et al. 2014). In 2015 the average July temperature was 14.6°C and the July precipitation was 80.0 mm (Harris et al. 2014).

Bylot Island

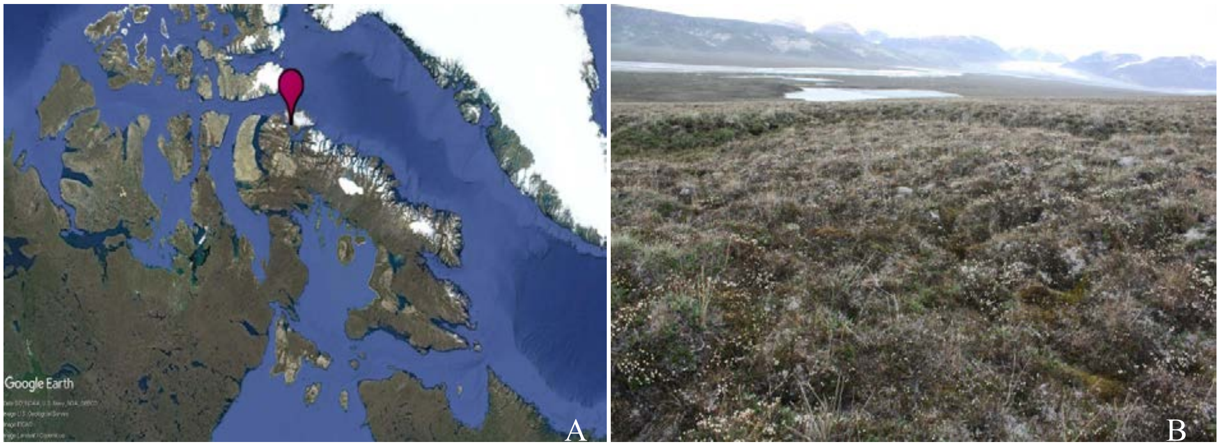

Figure 2.3 Bylot Island located north of Baffin Island, Nunavut, Canada (A), with graminoid tundra habitat (B). (Photo credit: Aurélie Chagnon-Lafortune).

The 'Bylot Island' site is located on the western coast of Bylot Island, Nunavut, Canada (73.15°N, 79.99°W). This site is located in a large glacial valley with mountains along both sides. All five plots are in located south of the Qarlikturvik Valley River which is fed by two large glaciers and flows into the Navy Board Inlet. The plots are roughly 5 km from the sea and range in elevation from 44-102 m a.s.l. Classified as graminoid tundra, this site consists of moderate (40-80%) vegetation cover (graminoid, prostrate dwarf-shrub, forb tundra, G2; CAVM 2003). The focal plant species for this site, by cover, are *Salix arctica, Cassiope tetragona, Arctagrostis latifolia, Oxyria digyna,* and *Papaver radicatum.* The site is located within bioclimatic subzone C (CAVM 2003), with an average July temperature and precipitation (1989-2012) of 7.3°C and 38.4 mm, respectively (Gauthier et al. 2013). In 2015 the average July temperature was 8.2°C while no data was available for the July precipitation that year (Harris et al. 2014). Bylot Island has been used in a long term study examining the trends in local wildlife populations and primary productivity, and examining how the corresponding changes in climate can explain the variation in these trends (Gauthier et al. 2013).

Pika Camp

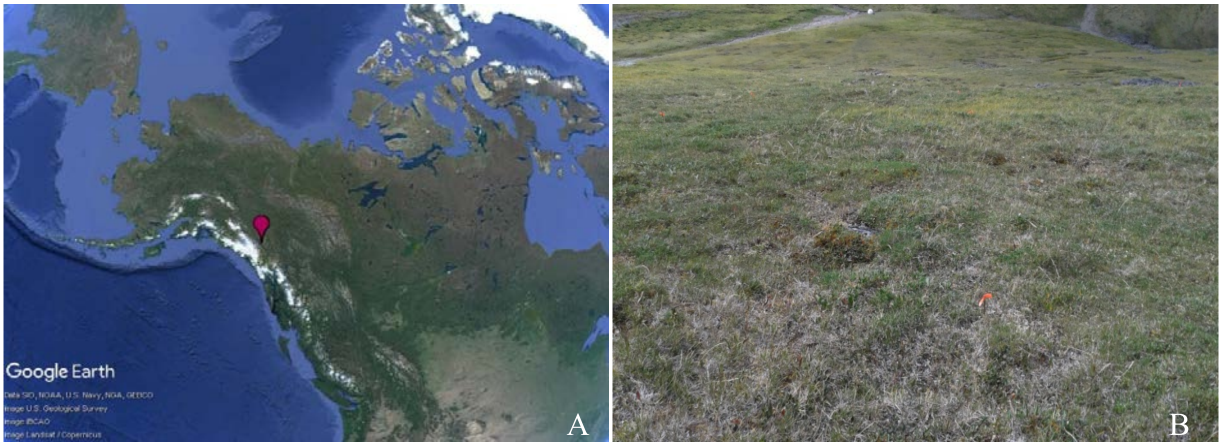

Figure 2.4 Pika Camp in SW Yukon, Canada (A), with Mesic tundra habitat (B). (Photo credit: Isabel C Barrio).

'Pika Camp' is located in an alpine valley in the Ruby Range in SW Yukon, Canada (61.22°N, 138.27°W). The five plots in this study site range in elevation from 1637-1774 m a.s.l. and are near Kluane Lake. Although the site is not included in the Circumpolar Arctic Vegetation Map, the dominant habitat corresponds to prostrate dwarf-shrub, herb tundra (P1; CAVM 2003), a dry tundra community dominated by *Dryas octopetala*, but vegetation cover is 100%. The focal plant species, by cover, are *Dryas octopetala, Carex bigelowii, Salix arctica,* and *Salix reticulata*. The site corresponds climatically to subzone E (CAVM 2003). Pika Camp has an average July temperature and precipitation (1990-2015) of 10.3°C and 58.0 mm, respectively (Harris et al. 2014). The July 2015 average temperature and total precipitation were 10.5°C and 80.4 mm, respectively (Harris et al. 2014). Previous studies at this site include foraging and population dynamics of small mammals (Morrison and Hik 2007, Patil et al. 2013, Wheeler and Hik 2014), as well as experiments examining how both vertebrate and/or invertebrate herbivory influence tundra vegetation (McIntire and Hik 2005, Barrio et al. 2013). Effects of increased temperature on invertebrate herbivory have also been examined here (Barrio et al. 2016).

FINLAND

Ailigas

Figure 2.5 Ailigas located in northern Finland (A), with dry alpine heath habitat (B). (Photo credit: Ilkka Syvänperä).

The site 'Ailigas' is located in northern Lapland, Finland (69.89°N, 27.07°E). Ailigas is a dry fjeld (high plateau above the treeline) located approximately 3 km south of the Teno River and 2.5 km southeast of Utsjoki village. A few small streams, ponds, and bogs are nearby and reindeer can be found grazing in this area. The five plots in this site range in elevation from 339-346 m a.s.l. Although the site is not included in the CAVM, the dominant habitat corresponds to erect-shrub tundra and consists of mostly continuous (90-100%) vegetation cover (erect dwarf-shrub tundra, S1; CAVM 2003). The focal plant species for this site, by cover, are *Betula nana, Empetrum nigrum,* and *Vaccinium vitis-idaea.* Ailigas corresponds to bioclimatic subzone E (CAVM 2003). The site corresponds to oroarctic tundra based on the classification by Virtanen et al. (2016). The average July temperature and precipitation (1990-2015) are 12.7°C and 77.8 mm, respectively (Harris et al. 2014). July 2015 had an average temperature of 10.5°C and a total precipitation of 32.7 mm (Harris et al. 2014). Studies conducted in this area have examined the phenology of plant root and leaf growth (Sloan et al. 2016) and range shifts in two tree species and the corresponding changes in demography (Matías and Jump 2015).

Njallavaara

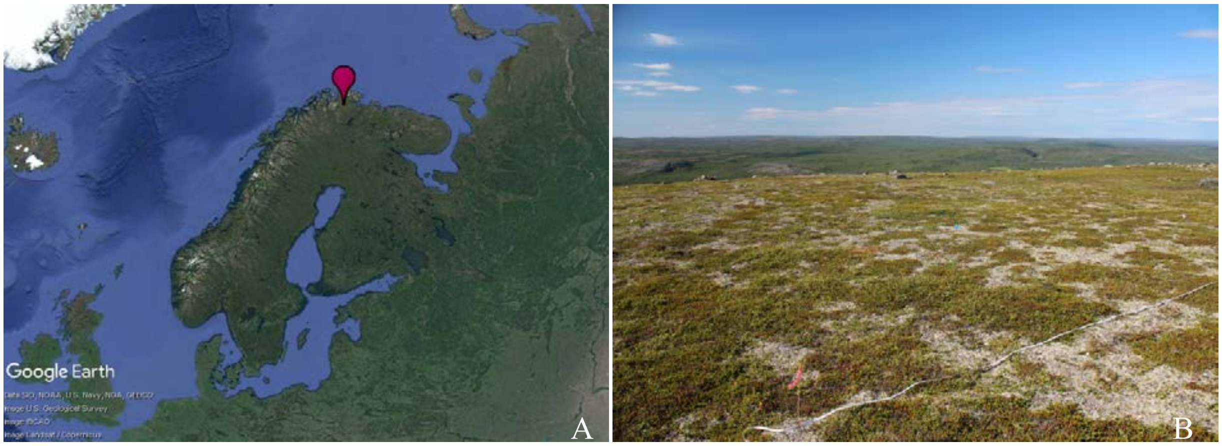

Figure 2.6 Njallavaara located in northern Finland (A), with dry alpine heath habitat (B). (Photo credit: Ilkka Syvänperä).

'Njallavaara' site is located in northern Lapland, Finland (70.04°N, 27.60°E), approximately 2.5 km southeast of the Teno River, where the elevation of the five plots ranges from 266-281 m a.s.l. Similar to Ailigas, Njalavaara is a dry fjeld with few water sources nearby and moderate reindeer presence. At the foot of the fjeld is the Teno river valley. Although the site is not included in the CAVM, the dominant habitat corresponds to erect-shrub tundra and consists of mostly continuous (90-100%) vegetation cover (erect dwarf-shrub tundra, S1; CAVM 2003). The focal plant species for this site, by cover, are *Vaccinium vitis-idaea, Empetrum nigrum,* and *Betula nana.* Njallavaara corresponds to bioclimatic subzone E (CAVM 2003) or oroarctic based on the classification by Virtanen et al. (2016). The average July temperature and precipitation (1990-2015) are 12.4°C and 70.9 mm, respectively (Harris et al. 2014). The average July temperature in 2015 was 10.3°C and the total precipitation was 27.2 mm (Harris et al. 2014). Studies conducted here have examined the effect of climate change on the reproductive success of an avian predator (Terraube et al. 2014), and whether insectivorous birds utilize olfactory cues from plants that are damaged by invertebrate herbivores (Mäntylä et al. 2014).

ICELAND

Audkuluheidi

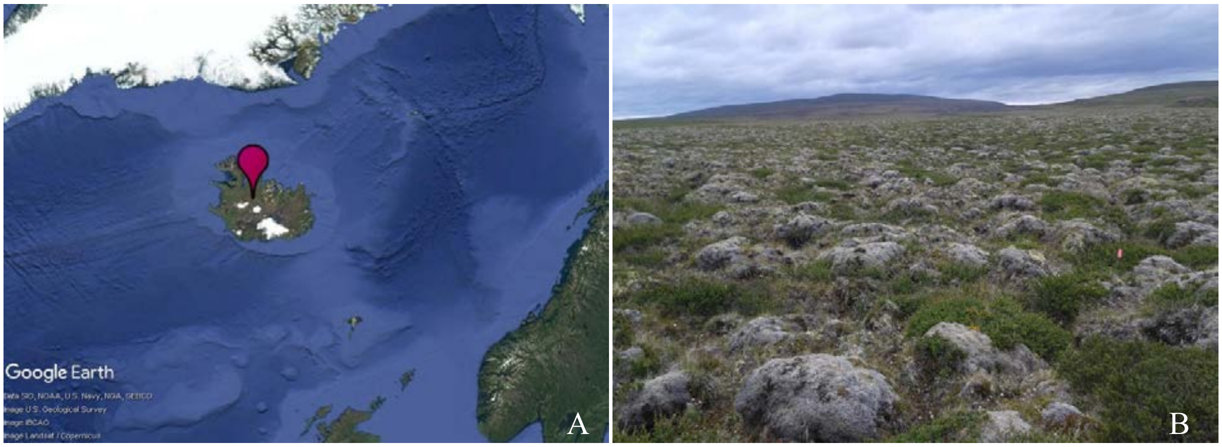

Figure 2.7 Audkuluheidi located in Northwest Iceland (A), with dwarf-shrub heath habitat (B). (Photo credit: Isabel C. Barrio).

'Audkuluheidi' is located in the central Highlands of Iceland (65.13°N, 19.67°W). Located south of the Blöndulón reservoir, the five alpine tundra plots in this site range in elevation from 479-498 m a.s.l. Although the site is not included in the Circumpolar Arctic Vegetation Map, the dominant vegetation type for this area corresponds to prostrate dwarf-shrub, herb tundra (P1; CAVM 2003), and consists of continuous vegetation cover. *Betula nana* and *Empetrum nigrum* were the two most dominant vascular plant species in all plots, but the third dominant species was not consistent across plots: *Vaccinium uliginosum,* and *Silene acaulis.* The site is located within bioclimatic subzone E (CAVM 2003), with an average July temperature and precipitation (1990-2015) of 10.2°C and 48.5 mm, respectively (Harris et al. 2014). The average temperature for July 2015 was 8.4°C and the total precipitation was 40.5 mm (Harris et al. 2014). This site was also used in a similar study that looked at the variation in invertebrate herbivory on dwarf birch species in tundra ecosystems (Barrio et al. 2017).

Fjallabak

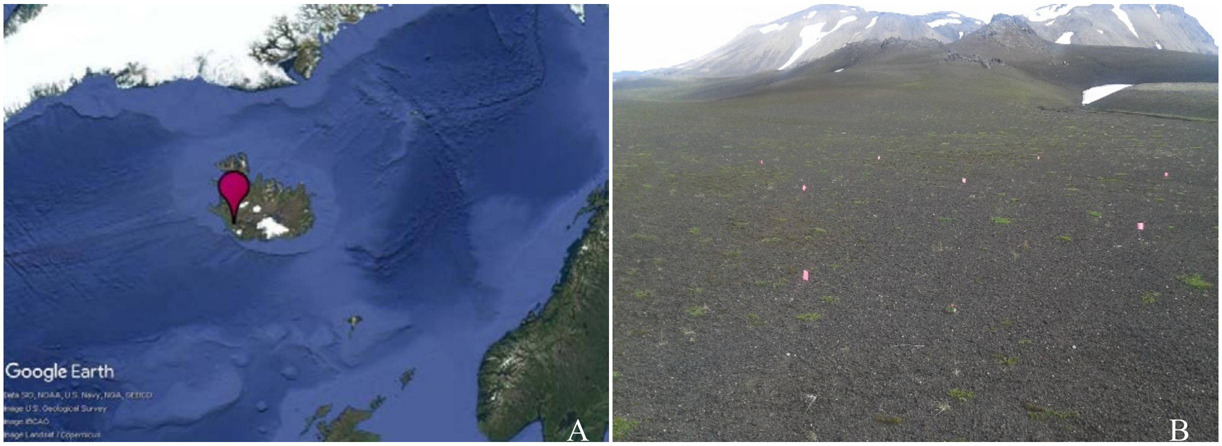

Figure 2.8 Fjallabak located in South Iceland (A), with barren habitat (B). (Photo credit: Isabel C. Barrio).

'Fjallabak' is located in South Iceland, approximately 17 km west of the Tindfjallajökull glacier (63.83°N, 19.91°W). Fjallabak is situated in the gravel fields that are associated with the Hekla volcano system. The five plots in this site range in elevation from 648-657 m a.s.l. The vegetation type for this area could be classified as cryptogam herb barren and consists of 10-20% vegetation cover (B1; CAVM 2003). *Salix herbacea* was the dominant vascular plant species in all plots, but the next dominant species were not consistent across plots: *Armeria maritima, Salix arctica, Oxyria digyna,* and *Cerastium alpinum.* The climate at the site would correspond to bioclimatic subzone E (CAVM 2003)**,** with an average July temperature and precipitation (1990-2015) of 9.9°C and 94.5 mm, respectively (Harris et al. 2014). In July 2015 the average temperature was 8.5°C and the total precipitation was 57.4 mm (Harris et al. 2014).

Skálpanes

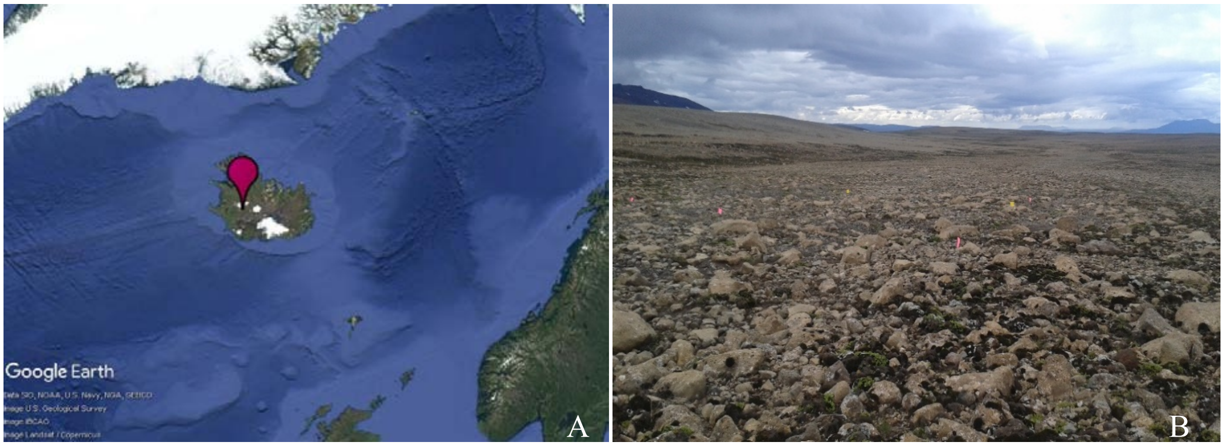

Figure 2.9 Skálpanes located in South Iceland (A), with barren habitat (B). (Photo credit: Isabel C. Barrio).

'Skálpanes' is located in South Iceland about 7 km southeast of the Langjökull glacier and 7 km southwest of Lake Hvítárvatn (64.52°N, 19.91°W). The site is located in a small mountain range close to the ocean and the five plots in this site range in elevation from 622-641 m a.s.l. The vegetation type for this area is classified as cryptogam herb barren and consists of 5-10% vegetation cover with majority of the area consisting of rocks (60%) and soil crust (40%) (B1; CAVM 2003). *Salix herbacea* and *Silene acaulis* were the two most dominant vascular plant species in all plots, but the third dominant species was not consistent across plots: *Armeria maritima, Juncus trifidus,* and *Luzula spicata.* The climate at the site corresponds to bioclimatic subzone E (CAVM 2003), with an average July temperature and precipitation (1990-2015) of 8.9°C and 66.6 mm, respectively (Harris et al. 2014). The average July 2015 temperature was 7.2°C and the total July precipitation was 49.0 mm (Harris et al. 2014).

Theistareykir

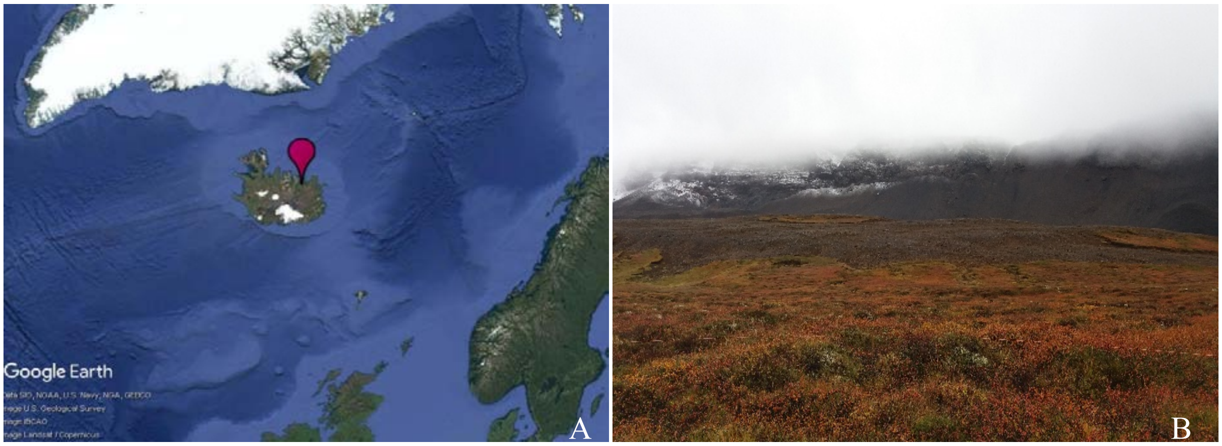

Figure 2.10 Theistareykir located in Northeast Iceland (A), with dwarf-shrub tundra habitat (B). (Photo credit: Isabel C. Barrio).

'Theistareykir' is located in Northeast Iceland approximately 19 km southeast of the coast of Skjálfandi Bay (65.90°N, 17.08°W). The five plots in this site range in elevation from 326-341 m a.s.l. The vegetation type for this area is classified as prostrate dwarf-shrub, herb tundra and has continuous vegetation cover (P1; CAVM 2003). The focal plant species for this site, by cover, are *Betula nana, Empetrum nigrum,* and *Calluna vulgaris.* The climate at the site corresponds to bioclimatic subzone E (CAVM 2003), with an average July temperature and precipitation (1990-2015) of 10.3°C and 54.8 mm, respectively (Harris et al. 2014). The average July 2015 temperature was 7.9°C and the total precipitation was 76.7 mm (Harris et al. 2014). This site was also used in a similar study that looked at the variation in invertebrate herbivory on dwarf birch species in tundra ecosystems (Barrio et al. 2017).

NORWAY

Hol

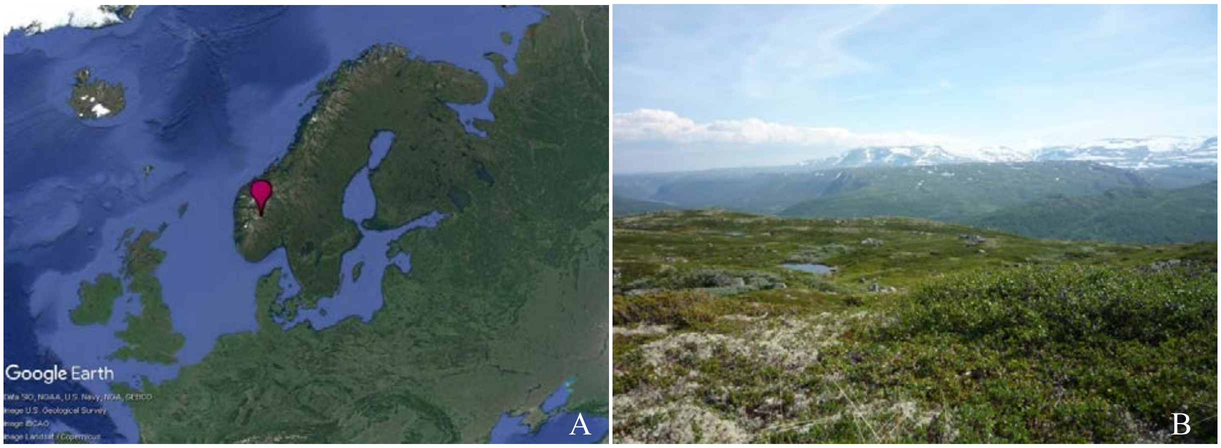

Figure 2.11 Hol located in Buskerud Norway (A), with mid-alpine shrub habitat (B). (Photo credit: James Speed).

The 'Hol' site is located in the Hol municipality in Buskerud, Norway (60.70°N, 7.94°E). The site is located approximately 2 km northeast of Lake Strandavatnet. Hol is a mountain site on a south facing slope above the birch forest treeline. This site was part of a long-term sheep grazing project and was therefore divided into nine plots: five plots with no sheep grazing and four plots with high sheep densities (Mysterud et al. 2010, Speed et al. 2012). Small lakes and streams can be found in the vicinity of the nine plots, which range in elevation from 1079-1200 m a.s.l. Although the site is not included in the CAVM, the dominant habitat corresponds to erect-shrub tundra and has mostly continuous (90-100%) vegetation cover (low-shrub tundra, S2; CAVM 2003). The focal plant species for this site, by cover, are *Betula nana, Vaccinium myrtillus, Empetrum nigrum, Avenella flexuosa, Vaccinium uliginosum,* and *Arctostaphylos uva-ursi.* Based on the definitions outlined by Virtanen et al. (2016) for the tundra sub-biomes Hol is classified as mid-latitude alpine tundra. Hol has an average July temperature and precipitation (1990-2015) of 11.9°C and 80.9 mm, respectively (Harris et al. 2014). In July 2015 the average temperature was 10.7°C and the total precipitation was 74.9 mm (Harris et al. 2014).

NORWAY (SVALBARD)

Bogstranda

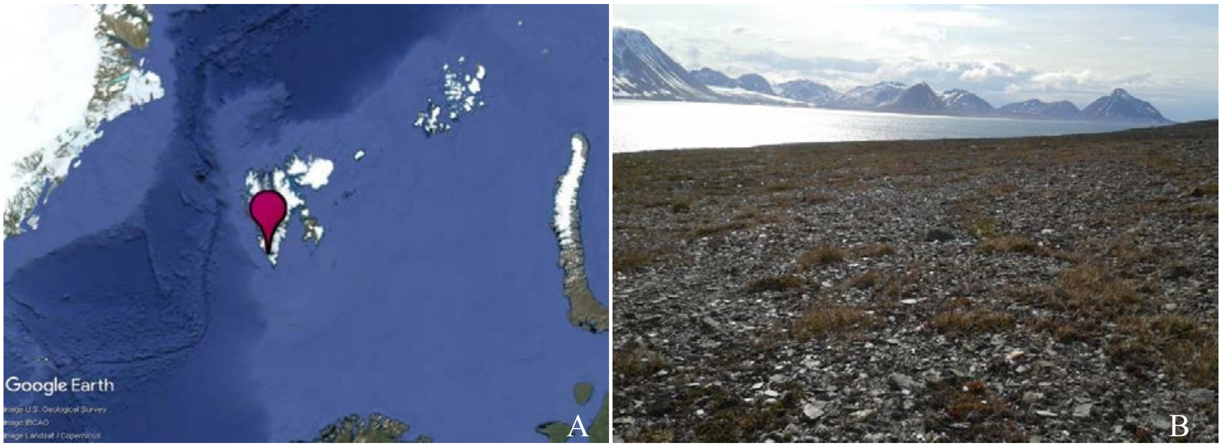

Figure 2.12 Bogstranda located in south western Svalbard (A), with dry tundra habitat (B). (Photo credit: Isabel C. Barrio).

'Bogstranda' is located in the southwest of Svalbard, approximately 0.5 km from the northern coast of the fjord Hornsund (77.02°N, 15.75°E). The five plots in this site range in elevation from 20-37 m a.s.l. The vegetation type for this site is classified as prostrate shrub tundra dominated by *Salix polaris* (prostrate dwarf-shrub, herb tundra P1; CAVM 2003). Vegetation cover in the plots ranged between 30-60%, with rocky surfaces exposed. The focal plant species, by cover, for this site are *Salix polaris, Saxifraga oppositifolia,* and *Festuca rubra.* The site is located within bioclimatic subzone B (CAVM 2003), with an average July temperature and precipitation (1990-2015) of 4.4°C and 46.0 mm, respectively (Harris et al. 2014). The average July 2015 temperature was 4.6°C and the total precipitation was 32.5 mm (Harris et al. 2014).

Kaffiøyra

Figure 2.13 Kaffiøyra located in western Svalbard (A), with prostrate dwarf-shrub, herb tundra (B). (Photo credit: Isabel C. Barrio).

'Kaffiøyra' is located approximately 1 km from the eastern coast of the sound Forlandsundet in western Svalbard (78.60°N, 12.24°E). The five plots in this site range in elevation from 27-31 m a.s.l. The vegetation type for this site is classified as prostrate-shrub tundra (prostrate dwarf-shrub, herb tundra, P1; CAVM 2003). Vegetation cover is moderate (30-50%) with mostly low growing plants (CAVM 2003). The focal plant species for this site, by cover, are *Dryas octopetala, Salix polaris, Silene acaulis, Saxifraga oppositifolia,* and *Bistorta vivipara.* The site is located within bioclimatic subzone A (CAVM 2003), with an average July temperature and precipitation (1990-2015) of 2.9°C and 53.1 mm, respectively (Harris et al. 2014). The average July 2015 temperature was 3.6°C and the total precipitation was 56.0 mm (Harris et al. 2014).

Kikutodden

Figure 2.14 Kikutodden located in southern Svalbard (A), with polar desert habitat (B). (Photo credit: Isabel C. Barrio).

'Kikutodden' is located at the southern tip of Sørkapp Land, approximately 0.6 km from the coast, in southern Svalbard (76.61°N, 16.96°E). The three plots in this site range in elevation from 11-18 m a.s.l. Kikutodden is a polar desert with the vegetation type classified as barren (cryptogam, herb barren, B1; CAVM 2003). Vegetation cover in the plots ranged between 20-50%, and was dominated by mosses and lichens. *Luzula confusa* was the dominant vascular plant species in all plots, but the next two dominant species were not consistent across plots: *Cerastium arcticum, Cochlearia groenlandica, Salix polaris, Saxifraga hyperborea,* and *Poa arctica.* The site is located within bioclimatic subzone B (CAVM 2003), with an average July temperature and precipitation (1990-2015) of 4.3°C and 48.2 mm, respectively (Harris et al. 2014). The average July 2015 temperature was 4.3°C and the total precipitation was 35.0 mm (Harris et al. 2014).

RUSSIA

Erkuta

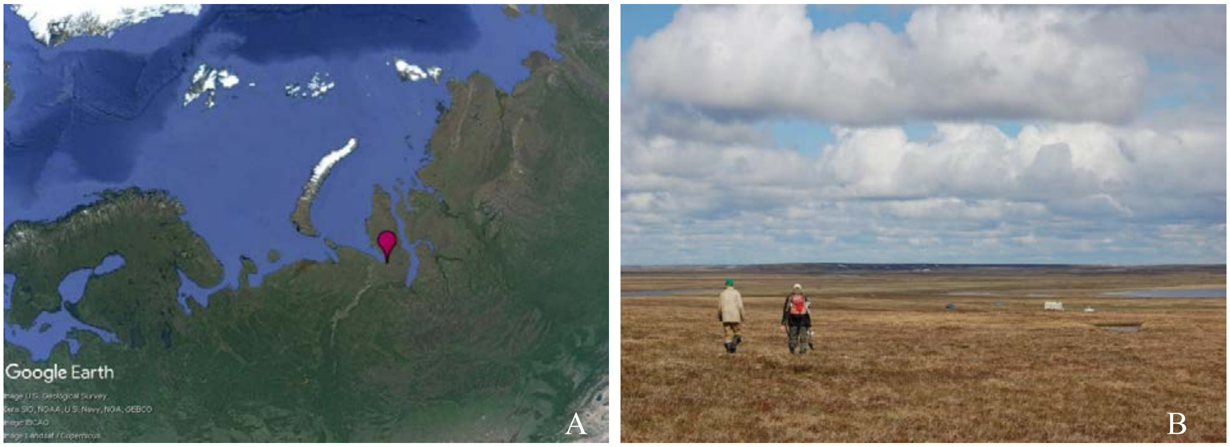

Figure 2.15 Erkuta tundra monitoring site located in the south of Yamal Peninsula, Russia (A), with a wetland habitat (B). (Photo credit: Dorothee Ehrich).

'Erkuta' is located in the Yamal-Nenets Autonomous District of Russia (68.23°N, 69.15°E). The site is located 28 km east of the coast of Baidarastkaya Bay, which is part of the Kara Sea. The five plots in this site were located on a flat low hill between the Payuta River and the Lake Mertsempertseto and have an elevation of ca 18 m a.s.l. The vegetation type for this area is classified as a sedge, moss, low-shrub wetland (W3; CAVM 2003). The focal plant species for this site, by cover, are *Betula nana, Carex* sp., and *Vaccinium vitis-idaea.* The site is located within bioclimatic subzone E (CAVM 2003), with an average July temperature and precipitation (1990-2015) of 12.9°C and 41.0 mm, respectively (Harris et al. 2014). In July 2015 the average temperature was 11.2°C and the total precipitation was 60.1 mm (Harris et al. 2014). Another study conducted in the same area examines the response of bird communities to climate change in tundra ecosystems, specifically how the ratio of generalist species to specialist species changes (Sokolov et al. 2012).

Murmansk

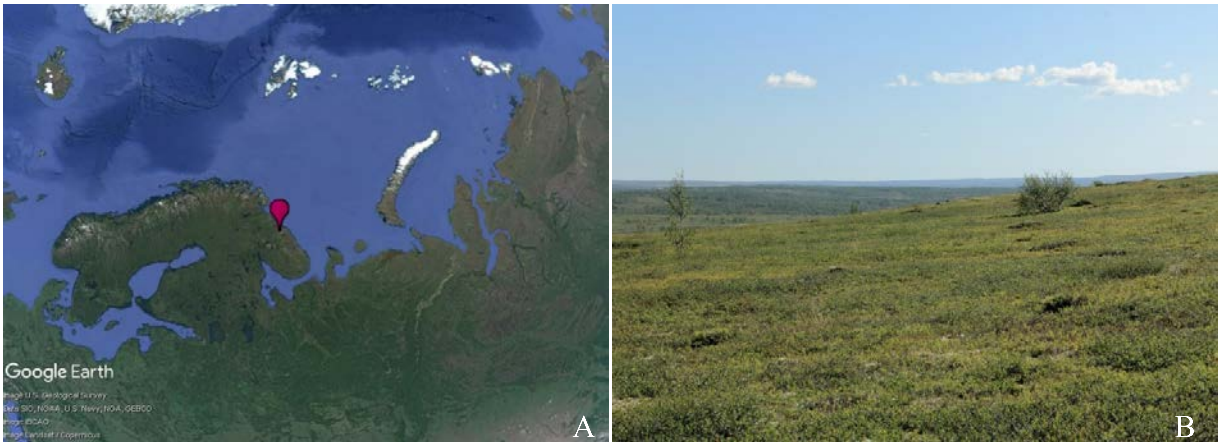

Figure 2.16 Murmansk located in northern Murmansk, Russia (A), with dwarf -shrub tundra habitat (B). (Photo credit: Vitali Zverev).

The 'Murmansk' site is located in the northern part of the Murmansk oblast, Russia (68.87°N, 34.54°E). The site is located on a north-facing slope of a small hill 60 km east of the city of Murmansk and 44 km south of the coast of the Barents Sea. The five plots in this site range in elevation from 246-265 m a.s.l. The vegetation type for this area is classified as erect-shrub tundra, and consists of continuous vegetation cover (erect dwarf-shrub tundra, S1; CAVM 2003). The focal plant species for this site, by cover, are *Betula nana, Empetrum nigrum,* and *Vaccinium myrtillus.* The site is located outside the Arctic, as defined by CAVM (2003). Murmansk has an average July temperature and precipitation (1990-2015) of 13.0°C and 69.3 mm, respectively (Harris et al. 2014). The average July 2015 temperature was 10.3°C and the total precipitation was 46.6 mm (Harris et al. 2014). This study site has been included in some multi-site studies such as one that looked the geographical variation in invertebrate herbivory in Europe (Kozlov 2008), and a study that looked at the circumpolar variation in invertebrate herbivory on dwarf birch species in tundra ecosystems (Barrio et al. 2017).

Severnaya

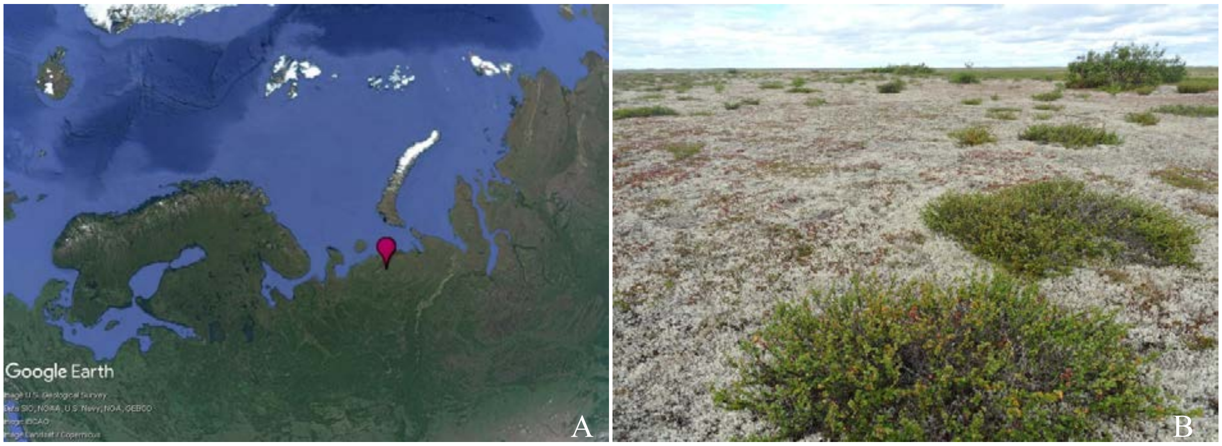

Figure 2.17 Severnaya located in northern Russia (A), with low-shrub tundra habitat (B). (Photo credit: Yulia V. Denisova and Sergey A. Uvarov).

'Severnaya' is located in the North-eastern part of the East European Plain in the Nenets Autonomous District of Russia (67.65°N, 54.04°E). Within the Severnaya River basin in the Bolshezemelskaya tundra the five plots ranged in elevation from 24-29 m a.s.l. Severnaya is in a large watershed between the Pechora River and its tributaries with mostly flat ground with some sandy hillocks and hollows. The vegetation type for this area is classified as erect-shrub tundra (Low-shrub tundra, S2; CAVM 2003). Total vegetative cover was approximately 90%. The focal plant species for this site, by cover, are *Betula nana, Arctous alpina,* and *Empetrum nigrum.* The site is located within bioclimatic subzone E (CAVM 2003), with an average July temperature and rainfall from 1990-2015 of 13.8°C and 55.7 mm, respectively (Harris et al. 2014). The average July temperature in 2015 was 10.0°C and the total July precipitation that year was 48.7 mm (Harris et al. 2014). Severnaya has also recently been used in a study examining the variation in invertebrate herbivory on dwarf birch species in tundra ecosystems (Barrio et al. 2017).

SWEDEN

Latnjajaure

Figure 2.18 Latnjajaure located in northern Sweden (A), with dwarf birch tundra habitat (B). (Photo credit: Peter Luptacik).

The study site 'Latnjajaure' is located in Norrbotten, Sweden (68.21°N, 18.29°E). The single plot is located approximately 1000 m a.s.l. in a mountain valley. Based on Virtanen et al. (2016), the vegetation type for this area is classified as low arctic dwarf birch tundra. The focal plant species for this site, by cover, are *Salix herbacea, Empetrum nigrum,* and *Betula nana.* This site is classified as being in the oroarctic tundra sub-zone (Virtanen et al. 2016). Latnjajaure has an average July temperature and rainfall (1990-2015) of 8.8°C and 102.8 mm, respectively (Harris et al. 2014). The average July temperature in 2015 was 7.1°C and the total July precipitation that year was 63.5 mm (Harris et al. 2014). This study site has been previously used in studies looking at how climate change can affect vascular plants (Alatalo et al. 2015) and lichens (Alatalo et al. 2017). Latnjajaure is also included as one of the long term sites in the International Tundra Experiment (Elmendorf et al. 2012).

Padjelanta

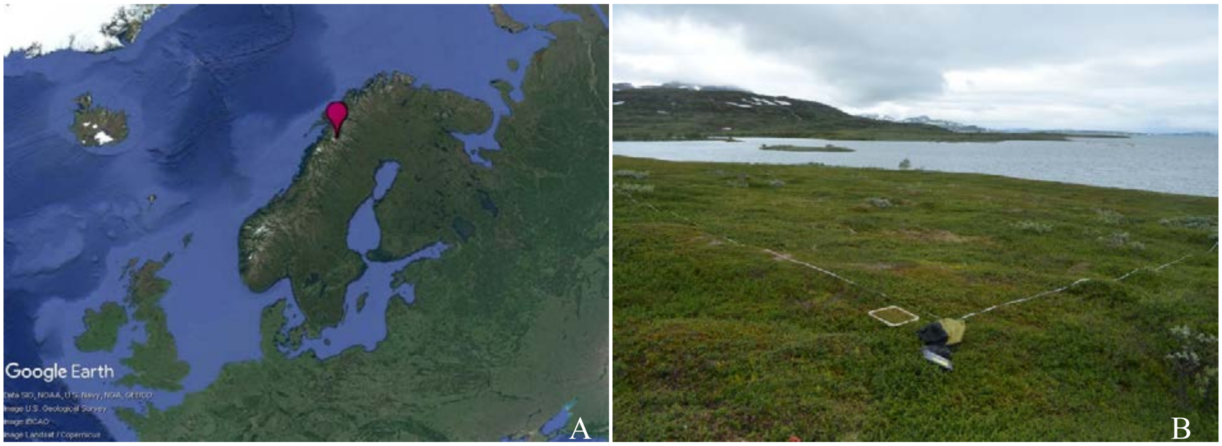

Figure 2.19 Padjelanta located in northern Sweden (A), with *Betula nana* heath habitat and Lake Virihaure in background (B). (Photo credit: Dagmar Egelkraut).

The site 'Padjelanta' is located in the Padjelanta National Park in Norrbotten, Sweden (67.31°N, 16.69°E). The study site is located just south of Lake Virihaure. Padjelanta is a hilly alpine environment with mires and lakes, and is a current and historical site for reindeer husbandry. The five plots range in elevation from 580-641 m a.s.l. The vegetation type for this area is classified as erect-shrub tundra (Erect dwarf-shrub tundra and low-shrub tundra, S1 and S2; CAVM 2003). *Betula nana* and *Empetrum nigrum* were the two most dominant vascular plant species in all plots, but the third dominant species varied between *Vaccinium vitis-idaea,* and *Vaccinium uliginosum*. The site would correspond to bioclimatic subzone E (CAVM 2003), with an average July temperature and precipitation (1990-2015) of 9.5°C and 106.4 mm, respectively (Harris et al. 2014). The average July 2015 temperature was 7.8°C and the total precipitation was 85.3 mm (Harris et al. 2014).

SWITZERLAND

Val Bercla

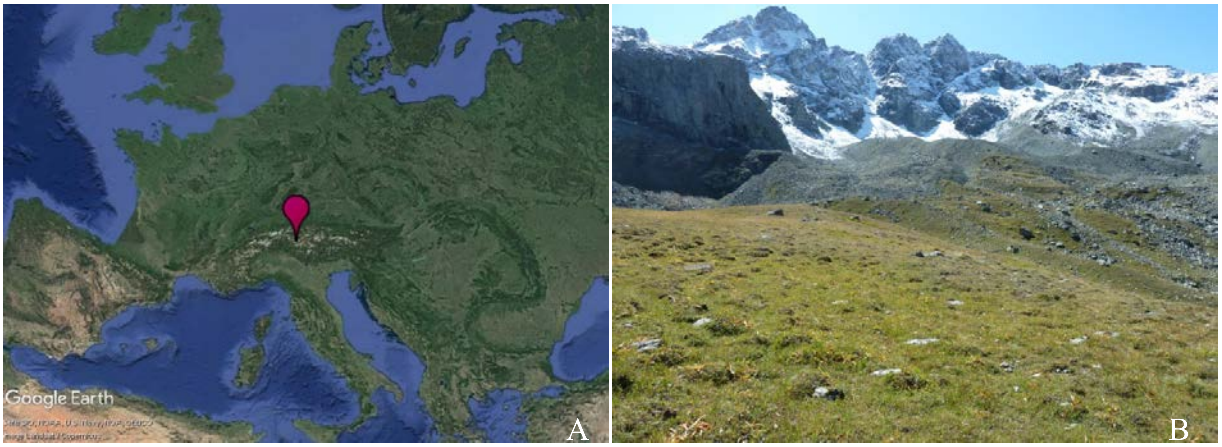

Figure 2.20 Val Bercla located in south east Switzerland (A), with alpine tundra habitat (B). (Photo credit: Janet Prevéy).

The study site 'Val Bercla' is in the central Alps of southeast Switzerland, approximately 0.5 km south west of the Val Bercla River (46.47°N, 9.58°E). The site is on a high alpine ridge with a north-northwest facing slope. The five plots have an elevation of 2490 m a.s.l. Val Bercla is an alpine tundra site and therefore is not included in the Circumpolar Arctic Vegetation Map (CAVM 2003). Vegetation cover in Val Bercla is roughly 55%. The focal plant species for this site, by cover, are *Primula integrifolia, Kalmia procumbens,* and *Helictochloa versicolor.* Val Bercla has an average July temperature and precipitation (1990-2015) of 8.9°C and 229.0 mm, respectively (Harris et al. 2014). The average July 2015 temperature was 12.1°C and the total July precipitation was 104.4 mm (Harris et al. 2014). Val Bercla is included in the International Tundra Experiment (Elmendorf et al. 2012). Some of the results from an ITEX experiment were published in a paper by Stenström et al. (1997), where they looked at how the phenology, growth, and reproduction of *Saxifraga oppositifolia* L. responded to simulated climate change at different latitudes, in which Val Bercla was used as the lowest latitude of the three sites.

UNITED KINGDOM

Mull

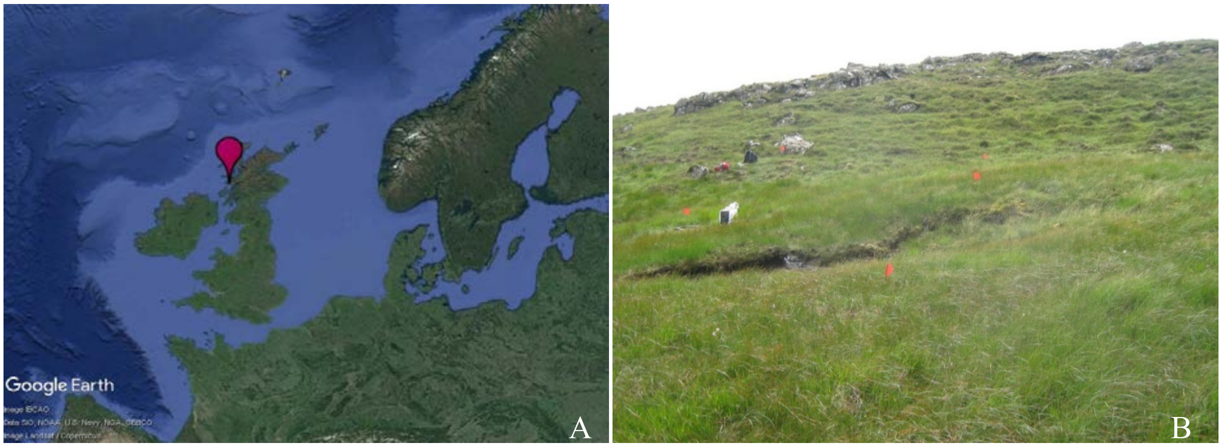

Figure 2.21 Mull located on the Isle of Mull, western Scotland, UK (A), with a graminoid habitat (B). (Photo credit: Francis Brearley).

The study site 'Mull' is located on the Isle of Mull in western Scotland, UK (56.37°N, 6.18°W). Mull is located on an easterly-facing slope with a small stream flowing along it. The single plot in this site has an elevation of approximately 400 m a.s.l. This site has a strong oceanic influence and is considered an outlier compared to the other sites. Due to this site not being considered an arctic site there is no CAVM classification for it. Mull had approximately 100% plant cover and the focal plant species for this site are *Trichophorum cespitosum, Calluna vulgaris,* and *Molinia caerulea.* The average July temperature and precipitation (1990-2015) were 14.0°C and 122.8 mm, respectively (Harris et al. 2014). In July 2015 the average temperature was 13.0°C and the total precipitation was 200.3 mm (Harris et al. 2014).

UNITED STATES OF AMERICA

Barrow

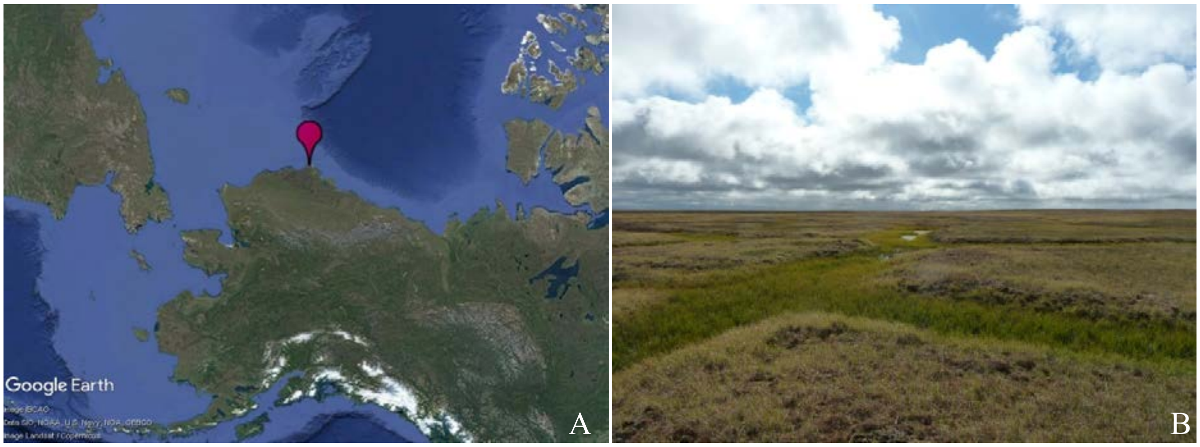

Figure 2.22 Barrow located in northern Alaska, USA (A), with wet meadow habitat (B). (Photo credit: Janet Prevéy).

The 'Barrow' site is located 2 km east of the city of Barrow and 2.5 km south of the coast in northern Alaska, USA (71.30°N, 156.67°W). Located in a wet meadow habitat, the five plots in this site have an elevation of approximately 10 m a.s.l. The vegetation type for this area is classified as wetland and has 95% vegetation cover (sedge/grass, moss wetland, W1; CAVM 2003). The focal plant species for this site, by cover, are *Carex aquatilis, Arctagrostis latifolia, Salix rotundifolia, Salix pulchra, Petasites frigidus,* and *Vaccinium vitis-idaea.* The site is located within bioclimatic subzone C (CAVM 2003), with an average July temperature and precipitation (1990-2015) of 5.3°C and 21.8 mm, respectively (Harris et al. 2014). In July 2015 the average temperature was 5.1°C and the total precipitation was 5.8 mm (Harris et al. 2014). Barrow is an active field site contributing to ITEX (Elmendorf et al. 2012). A recent study by Barrett and Hollister (2016) used data collected through ITEX to examine how long term warming affects plant growth and reproduction.

Toolik Lake

Figure 2.23 Toolik Lake located in northern Alaska, USA (A), with graminoid tundra habitat (B). (Photo credit: Ashley Asmus).

The study site 'Toolik Lake' is located roughly 1 km northeast of Toolik Lake in the northern foothills of the Brooks Range, in the North Slope region of Alaska, USA (68.64°N, 149.57°W). This site is near the Toolik Lake Field Station and the five plots are on a south-facing hillslope ranging in elevation from 730-746 m a.s.l. The vegetation type for this area is classified as graminoid tundra, and consists of 100% vegetation cover (tussock sedge, dwarf-shrub, moss tundra, G4; CAVM 2003). The focal plant species for this site, by cover, are *Betula nana, Rhododendron tomentosum,* and *Vaccinium vitis-idaea.* The site is located within bioclimatic subzone E (CAVM 2003), with an average July temperature and precipitation (1990-2015) of 11.6°C and 45.0 mm, respectively (Harris et al. 2014). The average July 2015 temperature was 11.4°C and the total precipitation was 38.6 mm (Harris et al. 2014). This study site has been used in research examining multitrophic interactions between plants, arthropods and birds (Boelman et al. 2014). Previous studies include a study examining the effect of shrub expansion on the abundance and diversity of arthropods (Rich et al. 2013), and a four year-long study by Sweet et al. (2015), that looked at the relationship between NDVI and canopy arthropod biomass.

Table 2.1 List of contributors to data collection for the 22 study sites.

Site	Contributors
Burntpoint Creek	Kim Bennet, Lisa Pollock, Rod Brook, Sophia Konieczka
Bylot Island	Guillaume Slevan-Tremblay, Gilles Gauthier, Esther Lévesque
Pika Camp	David Hik, Isabel Barrio
Ailigas	Otso Suominen, Tommi Andersson
Njallavaara	Tommi Andersson
Audkuluheidi	Isabel Barrio
Fjallabak	Isabel Barrio
Skálpanes	Isabel Barrio
Theistareykir	Isabel Barrio
Hol	James Speed
Bogstranda	Isabel Barrio, Ingibjörg Svala Jónsdóttir
Kaffiøyra	Isabel Barrio, Ingibjörg Svala Jónsdóttir
Kikutodden	Isabel Barrio, Ingibjörg Svala Jónsdóttir
Erkuta	Svetlana Abdulmanova, Capucine Baubin, Dorothee Ehrich, Natalya Sokolova, Alexander Sokolov
Murmansk	Mikhail Kozlov, Vitali Zverev
Severnaya	Sergey A. Uvarov, Yulia V. Denisova
Latnjajaure	Juha Alatalo, Miriam Rubin
Padjelanta	Dagmar Egelkraut, Johan Olofsson
Val Bercla	Janet Prévey
Mull	Francis Brearley
Barrow	Janet Prévey
Toolik Lake	Ashley Asmus

Table 2.2 Study site details. Includes the date in 2015 when samples were collected, the habitat type defined by CAVM (2003), and temperature and precipitation data from Harris et al. (2014). Symbols indicate when other sources of information were used, and details are given at the bottom of the table.

Study Site	Country	Collection Date	Habitat Type	Mean July Temperature (1990-2015) (°C)	Mean July Precipitation (1990-2015) (mm)	Mean July Temperature (2015) (°C)	Mean July Precipitation (2015) (mm)
Val Bercla	Switzerland	July 9	Alpine tundra	8.9	229.0	12.1	104.4
Fjallabak	Iceland	Aug 29	B1: Cryptogam, herb barren	9.9	94.5	8.5	57.4
Skálpanes	Iceland	Aug 15	B1: Cryptogam, herb barren	8.9	66.6	7.2	49.0
Kikutodden	Norway (Svalbard)	July 17	B1: Cryptogam, herb barren	4.3	48.2	4.3	35.0
Mull	United Kingdom	Aug 3	Coastal highland grassland	14.0	122.8	13.0	200.3
Bylot Island	Canada	July 16	G2: Graminoid, prostrate dwarf-shrub, forb tundra	7.3*	38.4*	8.2	--
Toolik Lake	USA	Aug 1	G4: Tussock-sedge, dwarf-shrub, moss tundra	11.6	45.0	11.4	38.6
Latnjajaure	Sweden	Aug 4	Low arctic dwarf birch tundra**	8.8	102.8	7.1	63.5
Pika Camp	Canada	July 27	P1: Prostrate dwarf-shrub, herb tundra	10.3	58.0	10.5	80.4
Audkuluheidi	Iceland	Aug 4	P1: Prostrate dwarf-shrub, herb tundra	10.2	48.5	8.4	40.5
Theistareykir	Iceland	Aug 2	P1: Prostrate dwarf-shrub, herb tundra	10.3	54.8	7.9	76.7
Bogstranda	Norway (Svalbard)	July 18	P1: Prostrate dwarf-shrub, herb tundra	4.4	46.0	4.6	32.5
Kaffiøyra	Norway (Svalbard)	July 14	P1: Prostrate dwarf-shrub, herb tundra	2.9	53.1	3.6	56.0
Ailigas	Finland	Aug 11-13	S1: Erect dwarf-shrub tundra	12.7	77.8	10.5	32.7
Njallavaara	Finland	Aug 20-21	S1: Erect dwarf-shrub tundra	12.4	70.9	10.3	27.2
Murmansk	Russia	Aug 11	S1: Erect dwarf-shrub tundra	13.0	69.3	10.3	46.6
Hol	Norway	July 17-20	S2: Low-shrub tundra	11.9	80.9	10.7	74.9
Severnaya	Russia	Aug 13	S2: Low-shrub tundra	13.8	55.7	10.0	48.7
Padjelanta	Sweden	Aug 2-3	S2: Low-shrub tundra	9.5	106.4	7.8	85.3
Barrow	USA	Aug 7-8	W1: Sedge/grass, moss wetland	5.3	21.8	5.1	5.8
Burntpoint Creek	Canada	June 25	W2: Sedge, moss, dwarf-shrub wetland	14.8	85.3	14.6	80.0
Erkuta	Russia	Aug 1-3	W3: Sedge, moss, low-shrub wetland	12.9	41.0	11.2	60.1

*1989-2012 (Gauthier et al. 2013) **Virtanen et al. (2016) -- No data available

Focal Species

The focal plant species that were found at least in six of the study sites are described in more detail here. The following species descriptions outline the growth form, leaf morphology, distribution, and information pertaining to responses/defenses to invertebrate herbivory.

Betula nana

Betula nana L. (dwarf birch) is a prostrate deciduous shrub that can grow up to 1m tall (de Groot et al. 1997). Leaves grow along the stem, are 8-15 mm long by 6-10 mm wide, dark green with a paler abaxial side, and have a serrated margin (Aiken et al. 2007; **Figure 2.24**). Two subspecies of *B. nana* have been recognized; *Betula nana* subsp. *nana* L., and *Betula nana* subsp. e*xilis* (Sukaczev) Hultén. These subspecies have chemical defenses to avoid herbivory including high concentrations of phenolic resins (especially in *B. nana exilis*) and terpens in young leaves that are generally more vulnerable (de Groot et al. 1997). These compounds have been shown to be toxic to many vertebrate herbivores (Bryant et al. 2014), but the effects on invertebrates have not been examined. However, based on results found by Barrio et al. (2017) the impact invertebrate herbivores have on *B. nana* does not differ between the two subspecies. *Betula nana* has a circumpolar distribution and can be found in Siberia, Alaska, and Northern Canada (subsp. *nana*), as well as in Iceland, and Northern Europe (subsp. *exilis*). Both subspecies overlap in Siberia and in Greenland (de Groot et al. 1997). *Betula nana* tends to grow in clumps and when growing in harsh environments (i.e. tundra), plants are low growing, have smaller leaves, and tend to rely more on asexual reproduction (de Groot et al. 1997). In this study *B. nana* samples were collected from 11 different study sites (**Table 2.3**).

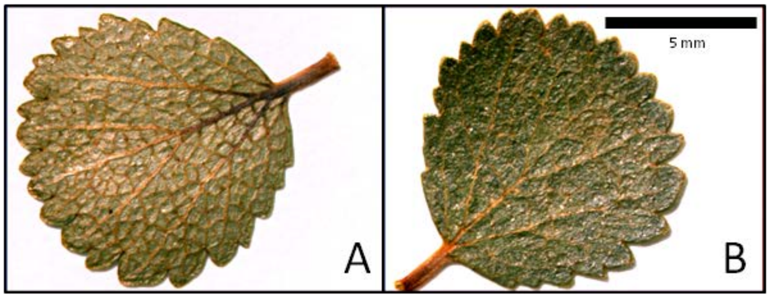

Figure 2.24 *Betula nana* leaf. Viewed from A: the abaxial side and B: the adaxial side

Empetrum nigrum

Empetrum nigrum L. (crowberry) is an evergreen dwarf shrub that can grow 5-35 cm high (Altan and Özdemir 2004, Aiken et al. 2007). *Empetrum nigrum* has extensive branching and forms dense mats that cover large areas (Bell and Tallis 1973, Altan and Özdemir 2004, Aiken et al. 2007). The leaves grow along the stems in a tight alternate pattern; leaves are oblong with a length of 2-6 mm and width of 0.5-1.5 mm (Altan and Özdemir 2004, Aiken et al. 2007; **Figure 2.25**). The leaf margins are strongly revolute, resulting in a deep furrow on the abaxial side created by the margins almost touching; the adaxial side is glabrous. The furrow on the abaxial side creates an air pocket and is covered with hairs (Bell and Tallis 1973, Altan and Özdemir 2004, Aiken et al. 2007). *Empetrum nigrum* is generally unpalatable to herbivores due to high levels of chemical defenses, including tannins, phenolic acids, and batatasin III. In green leaves >2% of the leaf dry weight was attributed to these compounds (Gallet et al. 1999). Therefore, any damage to its leaves during invertebrate outbreaks is likely due to starving larvae attempting to eat the leaves (Jepsen et al. 2013). The compounds found in *E. nigrum* leaves (especially batatasin III) have also been found to leach into the soil around the plant, resulting in changes in the soil conditions (Gallet et al. 1999). This allows *E. nigrum* to inhibit the growth of other plants and dominate in nutrient-poor forest understory habitats (Gallet et al. 1999, Olofsson et al. 2009, Jepsen et al. 2013). The distribution of *E. nigrum* is circumpolar with *E. nigrum* subsp. *nigrum* found across Northern Europe down to the Alps, Iceland, Russia, and Alaska; and *E. nigrum* subsp. *hermaphroditum* found in Alaska and Northern/alpine Canada (Bell and Tallis 1973). In this study *E. nigrum* is present as a focal species in 9 of the study sites (**Table 2.3**).

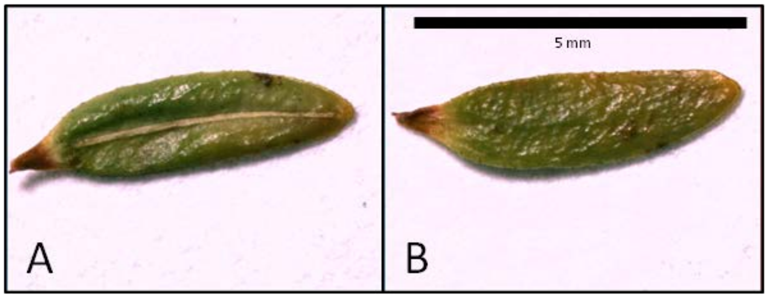

Figure 2.25 *Empetrum nigrum* leaf. Viewed from A: the abaxial side and B: the adaxial side.

Vaccinium vitis-idaea

Vaccinium vitis-idaea L. (lingonberry) is a dwarf evergreen shrub that grows in low mats that are 10-20 cm tall and have extensive branching (Ritchie 1955, Aiken et al. 2007). Leaves are 4-12 mm long and 3-8 mm wide. The blades are elliptic with an emarginate apex and a slightly revolute leaf margin (Ritchie 1955, Aiken et al. 2007). The adaxial surface is thick and dark green, while the abaxial surface is lighter and sparsely covered with small dark glands (Ritchie 1955; **Figure 2.26**). *Vaccinium vitis-idaea* has a circumpolar distribution and is found in Iceland, Northern Europe, Italy, Russia, Alaska, Northern Canada, and Greenland (Ritchie 1955). Whereas most evergreen shrubs tend to be avoided by invertebrate herbivores, MacLean and Jensen (1985) found that there were many species that still had a preference for consuming *V. vitis-idaea* leaves. In this study *V. vitis-idaea* was a focal species in 6 study sites (**Table 2.3**).

Figure 2.26 *Vaccinium vitis-idaea* leaf. Viewed from A: the abaxial side and B: the adaxial side.

Salix herbacea and Salix polaris

Although there are differences between these species, they were combined in order to increase the number of sites in this comparison. These species occur in similar communities, and have occasionally hybridized in the past (Beerling 1998). *Salix herbacea* L. (snowbed willow) and *S. polaris* Pall. (polar willow) are deciduous dwarf shrubs both of which produce dense mats formed from extensive underground rhizomes (Beerling 1998, Skarpe and van der Wal 2002, Hakkarainen et al. 2005, Aiken et al. 2007). For both species the aerial stems only just protrude from the mossy layer under which the rhizomes grow. The stems of *S. herbacea* grow 0.5-5 cm above the moss while those of *S.* polaris grow 1-9 cm above the moss (Beerling 1998, Skarpe and van der Wal 2002, Aiken et al. 2007). Leaf blades for both species are circular, obovate, or elliptic in shape. *S. herbacea* leaves are slightly smaller (6-20 mm long and 6-17 mm wide) than *S. polaris* leaves (5-32 mm long and 8-18 mm wide). The main difference between the leaves is the leaf margin; *S. herbacea* has a crenate margin (7-20 teeth per cm) and a shallowly emarginate apex, while *S. polaris* has an entire leaf margin with a rounded apex (Beerling 1998, Aiken et al. 2007). Lastly the leaves of *S. polaris* are slightly concave (Aiken et al. 2007; **Figure 2.27**). Both *S. herbacea* and *S. polaris* are dioecious, but the majority of reproduction is done via vegetative propagation (Beerling 1998, Skarpe and van der Wal 2002). *Salix herbacea* and *S. polaris* both have circumpolar distributions, and overlap in many areas. *Salix herbacea* ranges throughout Northern Canada, Alaska, Greenland, Iceland, Northern Europe, Svalbard, Russia, and mountainous parts of central Europe (Beerling 1998, Aiken et al. 2007), and *S. polaris* throughout Northern Canada, Alaska, Russia, Northern Europe, and Svalbard (Aiken et al. 2007). Instead of relying on chemical defenses to herbivore attacks *S. herbacea* and *S. polaris* rely on fast compensatory growth by activating dormant buds after damage has occurred (Skarpe and van der Wal 2002, Hakkarainen et al. 2005). In this study *S. herbacea* and *S. polaris* occur as focal species in a combined 6 study sites (3 and 3) (**Table 2.3**).

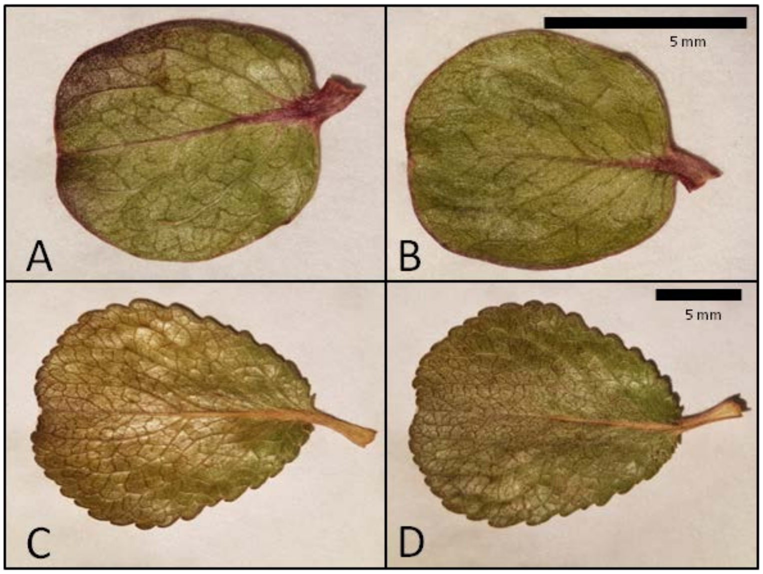

Figure 2.27 *Salix polaris* and *Salix herbacea* leaves. *S. polaris*, viewed from A: the abaxial side and B: the adaxial side. *S. herbacea* leaf, viewed from C: the abaxial side and D: the adaxial side.

Table 2.3 Plot information for each study site. The three focal species for each plot are included. Bolded species are the four target species used in the species level analysis. Sites that include a target species are indicated with an asterisk (*)

Study Site	Plot	Latitude	Longitude	Elevation (m.a.s.l.)	Focal Species		
Burntpoint Creek	1	55.243°	-84.318°	7	*Carex aquatilis*	*Trichophorum cespitosum*	*Andromeda polifolia*
	2	55.242°	-84.317°	8	*Carex aquatilis*	*Trichophorum cespitosum*	*Andromeda polifolia*
	4	55.241°	-84.316°	8	*Carex aquatilis*	*Trichophorum cespitosum*	*Andromeda polifolia*
	5	55.240°	-84.315°	8	*Carex aquatilis*	*Trichophorum cespitosum*	*Andromeda polifolia*
Bylot Island	1	73.148°	-79.988°	44	*Cassiope tetragona*	*Salix arctica*	*Arctagrostis latifolia*
	2	73.147°	-79.992°	46	*Cassiope tetragona*	*Salix arctica*	*Arctagrostis latifolia*
	3	73.145°	-79.990°	81	*Cassiope tetragona*	*Salix arctica*	*Oxyria digyna*
	4	73.143°	-79.994°	102	*Salix arctica*	*Arctagrostis latifolia*	*Papaver radicatum*
	5	73.145°	-79.999°	71	*Cassiope tetragona*	*Salix arctica*	*Arctagrostis latifolia*
Pika Camp	1	61.215°	-138.280°	1637	*Dryas octopetala*	*Salix arctica*	*Carex bigelowii*
	2	61.216°	-138.276°	1683	*Dryas octopetala*	*Salix reticulata*	*Carex bigelowii*
	3	61.217°	-138.275°	1709	*Dryas octopetala*	*Salix arctica*	*Carex bigelowii*
	4	61.218°	-138.273°	1750	*Dryas octopetala*	*Salix arctica*	*Carex bigelowii*
	5	61.217°	-138.271°	1774	*Dryas octopetala*	*Salix arctica*	*Carex bigelowii*
*Ailigas	1	69.895°	27.067°	346	***Betula nana***	***Empetrum nigrum***	***Vaccinium vitis-idaea***
	2	69.895°	27.064°	342	***Betula nana***	***Empetrum nigrum***	***Vaccinium vitis-idaea***
	3	69.895°	27.071°	339	***Betula nana***	***Empetrum nigrum***	***Vaccinium vitis-idaea***
	4	69.894°	27.076°	343	***Betula nana***	***Empetrum nigrum***	***Vaccinium vitis-idaea***
	5	69.894°	27.070°	346	***Betula nana***	***Empetrum nigrum***	***Vaccinium vitis-idaea***
*Njallavaara	1	70.046°	27.604°	278	***Betula nana***	***Empetrum nigrum***	***Vaccinium vitis-idaea***
	2	70.046°	27.607°	268	***Betula nana***	***Empetrum nigrum***	***Vaccinium vitis-idaea***
	3	70.047°	27.604°	268	***Betula nana***	***Empetrum nigrum***	***Vaccinium vitis-idaea***
	4	70.048°	27.602°	266	***Betula nana***	***Empetrum nigrum***	***Vaccinium vitis-idaea***
	5	70.048°	27.605°	281	***Betula nana***	***Empetrum nigrum***	***Vaccinium vitis-idaea***
*Audkuluheidi	1	65.133°	-19.674°	490	***Betula nana***	***Empetrum nigrum***	*Silene acaulis*
	2	65.133°	-19.674°	490	***Betula nana***	***Empetrum nigrum***	*Vaccinium uliginosum*

	3	65.133°	-19.674°	498	***Betula nana***	***Empetrum nigrum***	*Vaccinium uliginosum*
	4	65.133°	-19.674°	492	***Betula nana***	***Empetrum nigrum***	*Silene acaulis*
	5	65.133°	-19.674°	479	***Betula nana***	***Empetrum nigrum***	*Vaccinium uliginosum*
*Fjallabak	1	63.834°	-19.914°	657	***Salix herbacea***	*Armeria maritima*	*Cerastium alpinum*
	2	63.834°	-19.914°	648	***Salix herbacea***	*Armeria maritima*	*Oxyria digyna*
	3	63.834°	-19.914°	650	***Salix herbacea***	*Salix arctica*	*Armeria maritima*
	4	63.834°	-19.914°	649	***Salix herbacea***	*Armeria maritima*	*Oxyria digyna*
	5	63.834°	-19.914°	657	***Salix herbacea***	*Salix arctica*	--
*Skálpanes	1	64.524°	-19.914°	641	***Salix herbacea***	*Silene acaulis*	*Juncus trifidus*
	2	64.524°	-19.914°	622	***Salix herbacea***	*Silene acaulis*	*Juncus trifidus*
	3	64.524°	-19.914°	628	***Salix herbacea***	*Silene acaulis*	*Armeria maritima*
	4	64.524°	-19.914°	629	***Salix herbacea***	*Silene acaulis*	*Luzula spicata*
	5	64.524°	-19.914°	632	***Salix herbacea***	*Silene acaulis*	*Armeria maritima*
*Theistareykir	1	65.897°	-17.083°	338	***Betula nana***	***Empetrum nigrum***	*Calluna vulgaris*
	2	65.897°	-17.083°	341	***Betula nana***	***Empetrum nigrum***	*Calluna vulgaris*
	3	65.897°	-17.083°	338	***Betula nana***	***Empetrum nigrum***	*Calluna vulgaris*
	4	65.897°	-17.083°	335	***Betula nana***	***Empetrum nigrum***	*Calluna vulgaris*
	5	65.897°	-17.083°	326	***Betula nana***	***Empetrum nigrum***	*Calluna vulgaris*
*Hol	E1	60.698°	7.935°	1171	***Betula nana***	***Empetrum nigrum***	*Arctostaphylos uva-ursi*
	E2	60.697°	7.934°	1156	***Betula nana***	*Vaccinium myrtillus*	***Empetrum nigrum***
	E3	60.699°	7.937°	1200	***Betula nana***	*Vaccinium myrtillus*	***Empetrum nigrum***
	E4	60.696°	7.936°	1147	***Betula nana***	*Vaccinium myrtillus*	***Empetrum nigrum***
	E5	60.694°	7.935°	1079	***Betula nana***	*Vaccinium myrtillus*	*Avenella flexuosa*
	F1**	60.694°	7.940°	1092	***Betula nana***	*Vaccinium myrtillus*	*Vaccinium uliginosum*
	F2**	60.695°	7.941°	1112	***Betula nana***	*Vaccinium myrtillus*	*Avenella flexuosa*
	F3**	60.695°	7.941°	1128	***Betula nana***	*Vaccinium myrtillus*	*Avenella flexuosa*
	F4**	60.696°	7.942°	1147	***Betula nana***	*Vaccinium myrtillus*	*Avenella flexuosa*
*Bogstranda	1	77.018°	15.750°	37	***Salix polaris***	*Saxifraga oppositifolia*	*Festuca rubra*
	2	77.017°	15.754°	33	***Salix polaris***	*Saxifraga oppositifolia*	*Festuca rubra*
	3	77.017°	15.751°	37	***Salix polaris***	*Saxifraga oppositifolia*	*Festuca rubra*

	4	77.016°	15.750°	28	***Salix polaris***	*Saxifraga oppositifolia*	*Festuca rubra*
	5	77.015°	15.749°	20	***Salix polaris***	*Saxifraga oppositifolia*	*Festuca rubra*
*Kaffiøyra	1	78.599°	12.245°	28	*Dryas octopetala*	***Salix polaris***	*Silene acaulis*
	2	78.599°	12.239°	31	*Saxifraga oppositifolia*	***Salix polaris***	*Silene acaulis*
	3	78.600°	12.235°	27	*Dryas octopetala*	***Salix polaris***	*Silene acaulis*
	4	78.600°	12.229°	27	*Bistorta vivipara*	***Salix polaris***	*Silene acaulis*
	5	78.598°	12.253°	30	*Silene acaulis*	***Salix polaris***	*Dryas octopetala*
*Kikutodden	1	76.609°	16.958°	18	*Luzula confusa*	*Cochlearia groenlandica*	*Poa arctica*
	2	76.608°	16.958°	17	*Luzula confusa*	***Salix polaris***	*Cerastium arcticum*
	3	76.608°	16.962°	11	*Luzula confusa*	*Saxifraga hyperborea*	*Cerastium arcticum*
*Erkuta	1	68.227°	69.151°	18	***Betula nana***	***Vaccinium vitis-idea***	*Carex sp.*
	2	68.227°	69.151°	18	***Betula nana***	***Vaccinium vitis-idea***	*Carex sp.*
	3	68.227°	69.151°	18	***Betula nana***	***Vaccinium vitis-idea***	*Carex sp.*
	4	68.227°	69.151°	18	***Betula nana***	***Vaccinium vitis-idea***	*Carex sp.*
	5	68.227°	69.151°	18	***Betula nana***	***Vaccinium vitis-idea***	*Carex sp.*
*Murmansk	1	68.87°	34.548°	258	***Betula nana***	***Empetrum nigrum***	*Vaccinium myrtillus*
	2	68.872°	34.543°	251	***Betula nana***	***Empetrum nigrum***	*Vaccinium myrtillus*
	3	68.872°	34.541°	246	***Betula nana***	***Empetrum nigrum***	*Vaccinium myrtillus*
	4	68.870°	34.542°	265	***Betula nana***	***Empetrum nigrum***	*Vaccinium myrtillus*
	5	68.870°	34.544°	260	***Betula nana***	***Empetrum nigrum***	*Vaccinium myrtillus*
*Severnaya	1	67.641°	54.078°	24	***Betula nana***	*Arctous alpina*	***Empetrum nigrum***
	2	67.645°	54.045°	29	***Betula nana***	*Arctous alpina*	***Empetrum nigrum***
	3	67.647°	54.042°	29	***Betula nana***	*Arctous alpina*	***Empetrum nigrum***
	4	67.647°	54.04°	28	***Betula nana***	*Arctous alpina*	***Empetrum nigrum***
	5	67.646°	54.037°	28	***Betula nana***	*Arctous alpina*	***Empetrum nigrum***
*Latnjajaure	1	68.21°	18.29°	1000	***Salix herbacea***	***Empetrum nigrum***	***Betula nana***
*Padjelanta	1	67.312°	16.693°	637	***Betula nana***	***Empetrum nigrum***	***Vaccinium vitis-idaea***
	2	67.312°	16.695°	641	***Betula nana***	***Empetrum nigrum***	*Vaccinium uliginosum*
	3	67.317°	16.682°	584	***Betula nana***	***Empetrum nigrum***	***Vaccinium vitis-idaea***
	4	67.316°	16.677°	580	***Betula nana***	***Empetrum nigrum***	***Vaccinium vitis-idaea***

	5	67.318°	16.706°	628	***Betula nana***	***Empetrum nigrum***	*Vaccinium uliginosum*		
Val Bercla	1	46.47°	9.58°	2490	*Primula integrifolia*	*Kalmia procumbens*	*Helictochloa versicolor*		
	2	46.47°	9.58°	2490	*Primula integrifolia*	*Kalmia procumbens*	*Helictochloa versicolor*		
	3	46.47°	9.58°	2490	*Primula integrifolia*	*Kalmia procumbens*	*Helictochloa versicolor*		
	4	46.47°	9.58°	2490	*Primula integrifolia*	*Kalmia procumbens*	*Helictochloa versicolor*		
	5	46.47°	9.58°	2490	*Primula integrifolia*	*Kalmia procumbens*	*Helictochloa versicolor*		
Mull	1	56.369°	-6.181°	400	*Trichophorum cespitosum*	*Calluna vulgaris*	*Molinia caerulea*		
*Barrow	1	71.3°	-156.67°	10	*Salix rotundifolia*	*Arctagrostis latifolia*	*Carex aquatilis*		
	2	71.3°	-156.67°	10	*Salix pulchra*	*Arctagrostis latifolia*	*Carex aquatilis*		
	3	71.3°	-156.67°	10	*Salix pulchra*	*Arctagrostis latifolia*	*Carex aquatilis*		
	4	71.3°	-156.67°	10	*Salix rotundifolia*	*Petasites frigidus*	*Carex aquatilis*		
	5	71.3°	-156.67°	10	*Salix pulchra*	***Vaccinium vitis-idea***	*Carex aquatilis*		
*Toolik Lake	1	68.644°	-149.573°	738	***Betula nana***	*Rhododendron tomentosum*	***Vaccinium vitis-idaea***	*Eriophorum vaginatum*	*Carex bigelowii*
	2	68.644°	-149.571°	746	***Betula nana***	*Rhododendron tomentosum*	***Vaccinium vitis-idaea***	*Eriophorum vaginatum*	*Carex bigelowii*
	3	68.642°	-149.573°	735	***Betula nana***	*Rhododendron tomentosum*	***Vaccinium vitis-idaea***	*Eriophorum vaginatum*	*Carex bigelowii*
	4	68.643°	-149.575°	730	***Betula nana***	*Rhododendron tomentosum*	***Vaccinium vitis-idaea***	*Eriophorum vaginatum*	*Carex bigelowii*
	5	68.645°	-149.571°	746	***Betula nana***	*Rhododendron tomentosum*	***Vaccinium vitis-idaea***	*Eriophorum vaginatum*	*Carex bigelowii*

**High density sheep

Chapter 3: Hiding in the background: species and community-level patterns in background invertebrate herbivory in the tundra biome

Introduction

The current understanding of invertebrate herbivory in tundra environments is based on only a few studies (Kozlov et al. 2015b, Barrio et al. 2017). This lack of attention may be due to an assumption that the average damage caused by invertebrates has a negligible effect compared with the impact from larger vertebrate herbivores (Crawley 1989, Kotanen and Rosenthal 2000). Current trends associated with rapid climate change at high latitudes indicate that the tundra biome will continue to experience increased temperature and precipitation (Huntington et al. 2005, Post et al. 2009, IPCC 2013). Studies predict that the effect of increased temperatures on invertebrate herbivores may result in herbivore range expansion and increased levels of herbivory, even at background levels (Wolf et al. 2008, Kozlov et al. 2015b, Birkemoe et al. 2016). As well, changes in precipitation could affect the amount of damage caused by invertebrate herbivores indirectly, through their influence on leaf traits, such as leaf toughness (based on the structural materials that make up the leaf). For example, lower levels of precipitation can increase the toughness of leaves, thus decreasing their palatability for insect herbivores (Onoda et al. 2011).

Further, the Latitudinal Herbivory Hypothesis (Coley and Barone 1996), predicts that at higher latitudes the incidence of herbivory will be reduced. Thus, changes in the intensity of herbivory at higher latitudes as a result of changes in climate, may have a disproportionate effect. In order to assess these potential changes in invertebrate herbivory it is necessary to establish the baseline conditions to which spatial and temporal dynamics can be compared. By understanding the prevalence, extent, and variation in tundra invertebrate herbivory we can begin to establish that baseline. To determine the variation in herbivory a detailed study, consistently replicated across a large spatial scale including different habitat types is needed (Fraser et al. 2013, Sternberg and Yakir 2015). Understanding the sources of variation in patterns of invertebrate herbivory, and the spatial scale of its variation, makes it possible to design common protocols to facilitate effective monitoring of changes in background invertebrate herbivory.

In this chapter, the patterns in invertebrate herbivory in the tundra are examined at both a plant species level and at the plant community level, across 22 tundra sites from Eurasia and North America. Different spatial scales are included in the investigation, ranging from individual plants, sampling plots, and study sites. The species level analysis included four common dwarf shrub species, *Betula nana*, *Vaccinium vitis-idaea*, *Empetrum nigrum* and *Salix polaris/herbacea* (see **Chapter 2**). The community level analysis examined the biomass lost to herbivory for each study site using community weighted estimates, thus controlling for variation in plant community composition. We predict that species level variation in herbivory will be positively attributed to increases in July temperature and precipitation, and decreases in latitude for three of the target plant species (*B. nana*, *V. vitis-idaea*, and *S. polaris/herbacea*). For *E. nigrum* we expect little variation regardless of climatic factors due to the low palatability of the species (MacLean Jr. and Jensen 1985). At the community level, we predict that sites with higher July temperatures, higher precipitation, and at lower latitudes will show higher levels of invertebrate herbivory. In our analyses, we also included collection date as a potential confounding variable. Differences in collection date can show accumulation in herbivore damage, or, on the other hand, later sample collection could result in recording lower herbivory levels due to early leaf abscission triggered by herbivore damage (Zvereva and Kozlov 2014).

Methods

Study design

This study was conducted during the summer of 2015 on a circumpolar scale, involving 22 arctic/alpine tundra study sites (**Figure 2.1**; see Chapter 2 for a detailed description of the study sites). In order to keep data collection consistent across all sites, a common protocol designed by *The Herbivory Network* (**Appendix 1**) was used. The protocol aimed at collecting plant materials using a hierarchical design from individual plants to plots within each study site (**Figure 3.1**). The protocol was sent to members of the Herbivory Network resulting in participation from study sites selected by researchers in areas adjacent to their own research sites. A total of 30 researchers were involved in data collection and coordination at field sites (**Table 2.1**). The specific date when samples were collected was suggested to coincide with the peak of growing season, therefore due to variation in phenology collection ranged from June 25 - August 29.

A study site was broadly defined as an expanse of area 0.25-25 km^2 in which the sampling was conducted. The study sites were located in the tundra biome, which was broadly defined as being void of trees. Within the biome, sites belonged to either the oroarctic or alpine sub-biomes as defined by Virtanen et al. (2016). The most common/typical habitat type within each site was identified, so that study sites would represent a variety of habitats characteristic of the tundra biome as a whole and not of a particular habitat across tundra (**Table 2.2**); thus, the specific habitat type of each study site varied from moist tussock tundra to polar desert. Habitat types at each site were considered the dominant habitat type at a site not influenced by extremes in moisture, soil chemistry, or any disturbance. At each site, within the dominant habitat type, 5 plots, 20x20 m, were established ≥100 m apart from each other (**Figure 3.1**). The three most abundant plant species were identified in each plot based on their overall contribution to biomass, and recorded as the focal species (**Table 2.3; see Chapter 2**). The three focal species were thus plot specific and could differ between plots within the same study site. In total, the focal species included 45 different plant species (**Table 3.1**).

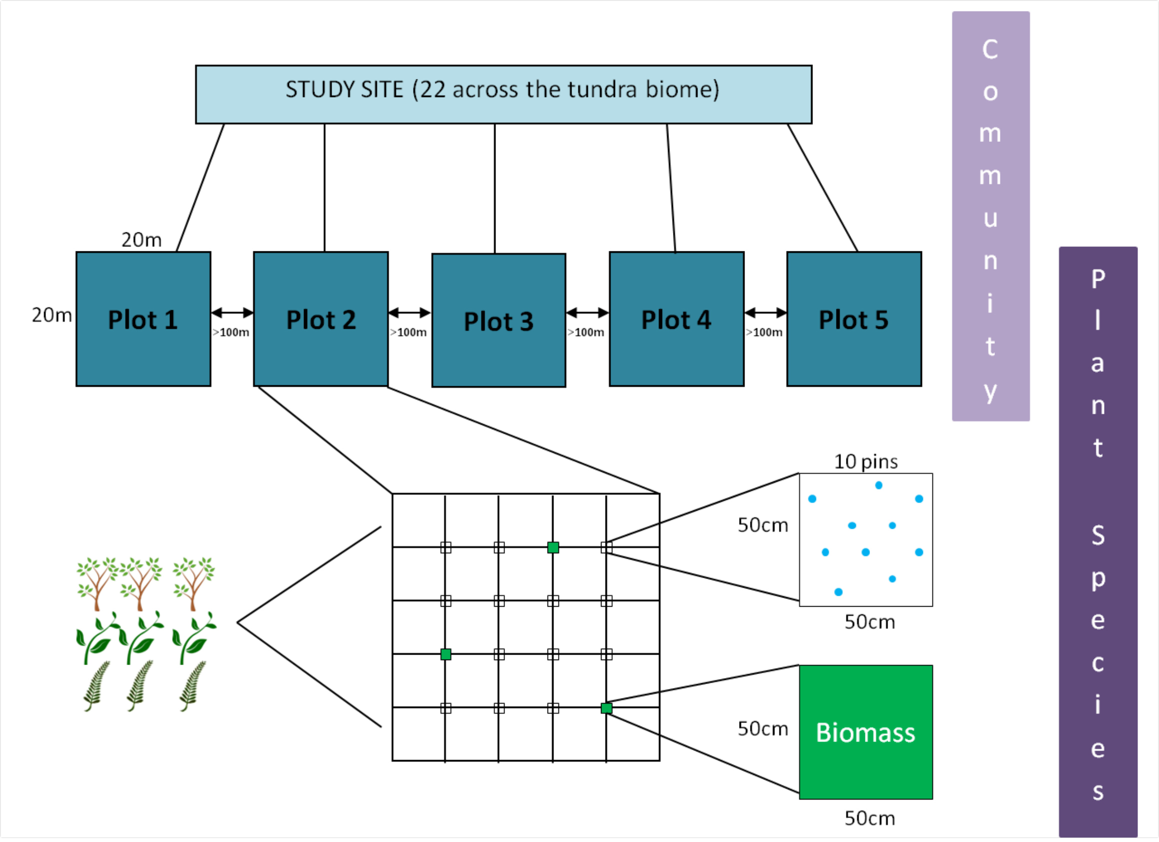

Figure 3.1 Visual representation of the study design breakdown. One large study site contained 5 plots that were 20 m x 20 m and separated by at least 100 m. Each plot was gridded evenly to have 16 frames, 5 m apart, that were 50 cm x 50 cm. Three focal species were identified at each plot; 100 leaves from three individuals of each focal species were taken and the point-intercept hits for each species were recorded for each frame. All above ground biomass was collected from three random frames in each plot.

Table 3.1 List of all 45 focal species and the total number of sites and plots they were found in. Included, as well, is the number of samples and the total number of leaves analysed for each species. In total 87,185 leaves were examined. Species taxonomy follows Roskov et al. (2017).

Focal Species	Growth Form	Number of Study Sites	Number of Plots	Number of Samples	Number of Leaves
Betula nana L.	Deciduous shrub	11	55	165	17764
Empetrum nigrum L.	Evergreen shrub	9	40	120	12456
Vaccinium vitis-idaea L.	Evergreen shrub	6	25	69	6935
Silene acaulis (L.) Jacq.	Herb	3	12	36	3602
Salix arctica Pall.	Deciduous shrub	3	11	23	2299
Salix herbacea L.	Deciduous shrub	3	11	33	3400
Salix polaris Wahlenb.	Deciduous shrub	3	11	33	3330
Vaccinium uliginosum L.	Deciduous shrub	3	6	18	1883
Vaccinium myrtillus L.	Deciduous shrub	2	13	39	3900
Carex bigelowii Torr.	Graminoid	2	10	30	2955
Carex aquatilis Wahlenb.	Graminoid	2	9	17	1666
Dryas octopetala L.	Shrub	2	8	23	2308
Arctagrostis latifolia (R. Br.) Griseb	Graminoid	2	7	7	692
Armeria maritima (Mill.) Willd	Herb	2	6	18	1802
Calluna vulgaris (L.) Hull	Evergreen shrub	2	6	16	1600
Saxifraga oppositifolia L.	Herb	2	6	17	1701
Trichophorum cespitosum (L.) Hartm.	Graminoid	2	5	13	1276
Oxyria digyna (L.) Hill	Herb	2	3	7	690
Arctous alpina (L.) Nied.	Deciduous shrub	1	5	15	1482
Carex sp. L.	Graminoid	1	5	15	1471
Eriophorum vaginatum L.	Graminoid	1	5	15	1471
Festuca rubra L.	Graminoid	1	5	15	1510
Helictochloa versicolor (Vill.) Romero Zarco	Graminoid	1	5	5	500
Kalmia procumbens (L.) Gift, Kron & P.F. Stevens ex Galasso, Banfi & F. Conti	Evergreen shrub	1	5	5	500
Primula integrifolia L.	Herb	1	5	5	437
Rhododendron tomentosum Harmaja	Evergreen shrub	1	5	15	1502
Andromeda polifolia L.	Evergreen shrub	1	4	12	1203
Avenella flexuosa (L.) Drejer	Graminoid	1	4	12	1256

Cassiope tetragona (L.) D. Don	Evergreen shrub	1	4	4	400
Luzula confusa Lindeberg	Graminoid	1	3	9	904
Salix pulchra Cham.	Deciduous shrub	1	3	3	293
Cerastium arcticum Lange	Herb	1	2	6	604
Juncus trifidus L.	Graminoid	1	2	6	600
Salix rotundifolia Trautv.	Deciduous shrub	1	2	2	200
Arctostaphylos uva-ursi (L.) Spreng.	Evergreen shrub	1	1	3	290
Bistorta vivipara (L.) Delarbre	Herb	1	1	3	195
Cerastium alpinum L.	Herb	1	1	3	299
Cochlearia groenlandica L.	Herb	1	1	3	300
Luzula spicata (L.) DC.	Graminoid	1	1	3	304
Molinia caerulea (L.) Moench	Graminoid	1	1	1	100
Papaver radicatum Rottb.	Herb	1	1	1	97
Petasites frigidus (L.) Fr. s.l.	Herb	1	1	1	104
Poa arctica R. Br.	Graminoid	1	1	3	300
Salix reticulata L.	Deciduous shrub	1	1	3	301
Saxifraga hyperborea R. Br.	Herb	1	1	3	303

Data collection

Leaf samples were collected from three individual plants for each of the three focal species at each plot. The definition of "individual" varied depending on the species, due to the prevalence of clonal growth in tundra ecosystems (Jonasson 1992); plants were considered different individuals when they were at least 10 m apart. In the case of plants that did not have enough leaves, either because the species had small stems or the individuals had few leaves, collection was done from multiple stems that were close together (e.g. within 1-2 m). In this case, leaf samples were collected from "aggregates". As suggested by Kozlov et al. (2014), the selection of individuals or aggregates was made from a distance of 5-10 m to avoid recognition of invertebrate herbivory during the selection process and avoid confirmation bias (i.e. picking individuals specifically because they were damaged). From each individual or aggregate at least 100 leaves were collected from different heights within the plant. Leaf samples were pressed as herbarium specimens and stored until analysis in the lab.

Above-ground biomass was collected using two complementary methods: point-intercept hits of focal species and harvesting living aboveground biomass. At each plot, 16 sampling points were placed in a regular grid 5 m apart (**Figure 3.1**). Point-intercept data were collected at each of the 16 sampling points within a plot, in most cases using a 50 cm x 50 cm frame (there was some variation in execution; **Table 3.2**) with ten fixed pin positions. For each of the focal species, the number of times a part of it touched one of the ten pins in a frame was recorded (i.e. multiple hits per pin per species). Three of the sampling points were randomly selected to collect total aboveground plant biomass using the same frame, after the point-intercept data were collected. The frames that had both point-intercept data and biomass collected were used to create an equation to allow for the estimation of plant biomass based on point intercept data for the rest of the frames in that plot (**Appendix 3**). Biomass samples were stored in paper bags and air-dried in the field until further processing. In the lab, biomass samples were sorted into the three focal species recorded for the corresponding plot, and ‘other’ biomass.

The above data collection methods were followed in their entirety at the majority of the study sites, although a few sites did make minor deviations from these methods as noted in **Table 3.2**.

Table 3.2 Deviations from the data collection protocol.

Site	Deviation	Protocol
	Frame size	
Theistareykir	Frame size 15 cm x 15 cm (biomass collection)	50 cm x 50 cm
Audkuluheidi	Frame size 15 cm x 15 cm (biomass collection)	50 cm x 50 cm
Val Bercla	Frame size 30 cm x 30 cm	50 cm x 50 cm
Padjelanta	Frame size 80 cm x 80 cm	50 cm x 50 cm
Bylot Island	Frame size 70 cm x 70 cm	50 cm x 50 cm
	Number of plots	
Kikutodden	3 plots	5 plots
Burntpoint Creek	4 plots	5 plots
Latnjajaure	1 plot	5 plots
Hol	9 plots	5 plots
Mull	1 plot	5 plots
	Number of individual plants sampled per plot	
Mull	1 individual sampled per plot	3 individuals sampled per plot
Barrow	1 individual sampled per plot	3 individuals sampled per plot
Val Bercla	1 individual sampled per plot	3 individuals sampled per plot
Bylot Island	1 individual sampled per plot	3 individuals sampled per plot
	Biomass sampling	
Bylot Island	Calculated biomass themselves	Biomass estimated via point-intercepts
Severnaya	Measured % cover of the focal species	Biomass estimated via point-intercepts
Erkuta	Biomass for FS1 calculated separately	Biomass estimated via point-intercepts
Toolik Lake	No biomass collected	Biomass estimated via point-intercepts
	Other deviations	
Toolik Lake	5 focal species per plot	3 focal species per plot
Hol	4 plots with high sheep densities	
Hol	Biomass only collected for high density sheep plots	Biomass collected for all plots
Hol	Biomass not sorted by focal species	Biomass sorted by focal species

Damage assessment

Leaf sample preparation involved separating the leaves from the branches/stems, or, for graminoids, from the base. For some species more than the necessary 100 leaves were provided (i.e. *Empetrum nigrum*). In these cases, leaves were systematically removed from branches so that the sample included some leaves from all the branches and all the different parts of the branch. A dissecting microscope was used to observe leaves for damage, due to the small size of leaves and invertebrate damage marks. Each leaf was examined on both sides with a light source shinning down on to the leaf to assess external damage, and then, both sides were examined with a light source shinning up through the leaf to evaluate internal damage. Leaves were sorted into three main categories; undamaged, damaged by invertebrate herbivores, and other damage. Invertebrate damage was categorized in to three main types, and recorded separately: damage caused by external feeders, mining, and gall damage (**Figure 1.1**). External damage included chewing damage and skeletonization. Chewed leaves had chunks of leaf tissue removed (**Figure 1.1A**); skeletonized leaves had the outer epidermis eaten while the inside layer of cells was left intact (**Figure 1.1B**). Internal damage by leaf miners included both window and serpentine mining. Mined leaves were defined as having both leaf epidermises intact while the inside of the leaf was consumed, the pattern in which this was done determined if the mine was serpentine (following a path; **Figure 1.1C**), or window (patch; **Figure 1.1D**). Gall damage included galls formed on either the abaxial or adaxial surface of the leaf (**Figure 1.1E**), or on the petiole (**Figure 1.1F**).

After the type of herbivory was determined, the percent area of the leaf that was damaged by invertebrates was estimated and classified into one of six levels. Based on a frequent method used in other studies, the levels were defined as <1%, 1-5%, 5-25%, 25-50%, 50-75%, and >75% (Kozlov 2008, Barrio et al. 2017). In the case that two different types of invertebrate herbivory were present on the same leaf the second damage type (smaller percentage) was recorded as secondary damage. Secondary damage was recorded such that the area lost was still included in the analysis but the leaf was not double counted in the total number of leaves.

Leaves classified as "other damage" showed damage that could not be clearly attributed to invertebrates (i.e. rips, fungus, necrosed tissue, and dark spots of unknown origin).

Assessment of leaf damage was repeated for ~100 leaves per individual. In some cases, leaves were discarded if they were folded during the drying process such that unfolding them would result in crushing the leaf, thus resulting in fewer than 100 leaves in that sample. This process was done for all 45 focal plant species, the resulting number of samples and leaves analysed per species can be seen in **Table 3.1**.

Calculation of response variables

Species-level analysis

In order to assess the variation in invertebrate herbivory levels within a species, the four most common focal species were selected (**Table 2.3**; see Chapter 2 for a description of the target species): *Betula nana* (found in 11 study sites), *Empetrum nigrum* (9 study sites), *Vaccinium vitis-idaea* (6 study sites), and the combination of the closely related *Salix herbacea* and *S. polaris* (6 study sites). For the species-level assessment three complementary measures of damage were calculated: percent leaves damaged, percent leaf area damaged, and average percent area damaged per leaf.

The percent leaves damaged (PLD) was calculated as the number of leaves in a sample that had signs of invertebrate damage divided by the total number of leaves in that sample. PLD was calculated for total damage, external damage, mining damage, and gall damage. PLD gives an idea of how widespread the damage is on a plant (i.e. how many leaves are affected).

The percent leaf area damaged (PLAD) was calculated as the average leaf area damaged per leaf. PLAD thus, gives a more accurate representation of the intensity of damage on a plant (i.e. how much of its foliar area is affected by damage). After sorting the damaged leaves based on damage type and level of damage, each leaf was assigned the median value for that damage bin. For example, a leaf in the 25-50% bin was assigned as having 37.5% damage. The median value for all leaves in a sample was then summed and divided by the total number of leaves in the sample. In the case that a leaf had two different types of damage the median value for both types were summed but the leaf was only counted once in the total number of leaves. PLAD was calculated for total damage, external damage, mining damage, and gall damage.

The average percent area damaged per damaged leaf (APAD) was determined in order to examine how much of a leaf is eaten after a herbivore has started feeding before moving to a different leaf. APAD is calculated by dividing the sum of the median values by the number of damaged leaves. APAD was also calculated for total damage, external damage, mining damage, and gall damage.

Community-level analysis

For the community level assessment, the community weighted biomass loss (CWB) was calculated for each plot (Eq. 1). The CWB was calculated taking into account the total biomass contributed by each of the focal species, and how much of this was consumed by invertebrates. CWB thus “removes” the effect of different species composition at different study sites, and allows for comparisons across sites with different habitat types. CWB is expressed here as a percentage of the total biomass of the focal species present at a plot to control for the variation in biomass between tundra sites with contrasting plant biomass, from polar deserts to shrub tundra.

$$CWB_i = \left(\frac{\sum_{j=1}^{3}\left(BM_{ij} * \overline{PLAD}_{ij}\right)}{\sum\left(BM_{FS_j}\right)}\right) * 100 \qquad \text{Eq. 1}$$

Eq. 1 Calculation of the community weighted biomass lost to invertebrate herbivores for a plot i was based on the biomass (BM) of each of the three focal species (FS_j), and the leaf area removed by invertebrate herbivores, as estimated with the percent leaf area damaged (PLAD).

In order to determine the CWB for each plot, the point-intercept data needed to first be converted to a biomass estimate. This conversion utilized a linear regression weighted by a conversion factor. The conversion factors were calculated for each individual species or in some cases (when not enough data was available) for groups of species, and involved the use of the collected biomass and the corresponding point-intercept data for that frame (**Appendix 3**). To obtain the conversion factor, the sum of the species biomass (for the frames that had biomass collected) was divided by the sum of the number of point-intercept hits divided by ten (ten due to there being ten pins; Eq. 2). The calculation of these conversion factors was based on Bråthen and Hagberg (2004).

$$CF_{FS_i} = \frac{\sum BM_{FS_i}}{\sum \frac{IH_{FS_i}}{10}} \qquad \text{Eq. 2}$$

Eq. 2 Conversion factor calculation for focal species i. BM = Biomass FS = Focal Species IH = Point Intercept Hits

Statistical analysis

Species-level analysis

The variation in herbivory for the four target species was analysed using a Linear Mixed Effects Model (LMM) (Zuur et al. 2009). Potential predictors included latitude, collection date, and two different temperature and precipitation measurements. Latitude was included because according to the latitudinal herbivory-defense hypothesis the diversity of herbivores decrease with latitude (Anstett et al. 2016). Collection date coincided with the peak growing season, but varied for each site. Therefore, collection date was included as a predictor variable to examine whether a later collection date can lead to accumulated damage or suggest early leaf abscission if, for example, fewer damaged leaves are found later in the season (Zvereva and Kozlov 2014). Temperature and precipitation data were compiled from the CRU TS3.10 Dataset (Harris et al. 2014), and divided into long term July means (based on data from 1990-2015) and 2015 July values. Long-term means were used in order to include the yearly variation in temperature and precipitation. The 2015 climate data was used to see how the climate during the collection year affected observed herbivore damage.

The predictor variables were analysed for collinearity and multicollinearity across all the sites with the four target species present (17 sites; **Table 2.3**) before selecting which variables to use in the analyses. Pairwise correlation between predictors was checked using Pearson's linear correlation coefficient. Variance inflation factors (VIFs) were also calculated for each predictor variable to assess multicollinearity. Based on the Pearson's coefficients, the correlation between the two climate variables (temperature and precipitation) was smaller for the long-term means ($r = 0.03$) than the 2015 values ($r = -0.18$). This, combined with the low correlation between long-term mean precipitation and collection date ($r = 0.18$) and latitude ($r = -0.28$), led to dropping the 2015 temperature and precipitation data as predictor variables. The remaining predictors all had VIFs less than 3, suggesting no multicollinearity problems. Consequently, the predictor variables used for *B. nana*, *E. nigrum*, and *V. vitis-idaea*, were latitude, collection date, long-term mean July temperature, and long-term mean July precipitation. For the subset of sites containing *S. polaris* and *S. herbacea* the predictor variables showed stronger correlations ($|r| = 0.81 - 0.99$), so only long-term mean July temperature was used.

In the models for each of the indices of herbivory (PLD, PLAD, and APAD) and each of the feeding groups (all herbivores, external feeders, miners and gall-makers), the predictor variables listed above were used as fixed effects, while study site and plots were included as random effects to account for the nestedness of the study design. Estimates of the effect of each predictor variable, together with their 95% confidence intervals are reported, as well as the percentage of variation attributed to the nested random effects.

Community level analysis

Similar to the species-level analysis, the community level analysis utilized a Linear Mixed Effects Model (Zuur et al. 2009) to determine the level of variation in invertebrate herbivory across the study sites. For this analysis the Severnaya plots were dropped due to inconsistencies in biomass estimation (**Table 3.2**); as well, Bylot Island was excluded because of an inconsistent climate data source (**Table 2.1**). The same potential predictors were analysed for collinearity (latitude, collections date, mean July temperature 2015, July precipitation 2015, long-term mean July temperature, and long-term mean July precipitation) across the subset of 20 study sites. Due to the high Pearson's correlation coefficient between latitude and the other predictors latitude was dropped (|r| = 0.26 (Date), 0.51 - 0.74). Collection date showed low correlation with all other predictors (|r| = 0.07 - 0.36) so it was retained for analysis. Lastly, the 2015 July temperature and precipitation data had a higher correlation between themselves (r = 0.45) compared with the long-term mean July temperature and precipitation data (r = 0.16). This led to excluding the 2015 climate predictors. The percent of biomass lost to invertebrate herbivores in each plot was compared using the long-term mean July temperature, the long-term mean July precipitation, and collection date as fixed effects. Study site was included as a random effect.

Model assumptions were checked by examining plots of the residuals versus fitted values to determine homoscedasticity of variances; normality of residuals was examined via QQ-plots. All statistical analyses were carried out in R 3.3.3 (R Development Core Team 2017), and linear mixed effects models were created using the lme4 package (Bates et al. 2015).

Results

Species-level herbivory

Betula nana

A total of 17,764 *Betula nana* leaves were examined (165 individuals), from 11 sites across the tundra biome. Of these leaves, 2,507 had some form of invertebrate herbivore damage (14.11%). External damage (chewed and skeletonized) occurred on 2,477 leaves (13.94%), mining damage on 18 leaves (0.10%), and 12 leaves (0.07%) had gall damage. Since so few leaves had mining or gall damage only PLD was calculated for these types of damage. The average number of leaves damaged per sample (individual plant) was 13.53 ± 1.26%, and the average leaf area lost per sample was 1.86 ± 0.25%.

The PLD by all herbivory types on *Betula nana* was positively influenced by mean July temperature, mean July precipitation, and latitude, while collection date had no effect. When examining the different types of herbivory separately, external damage was positively influenced by temperature and precipitation; mining damage was not influenced by any of the predictors; gall damage was positively influenced by increasing latitude and negatively influenced by later collection dates. Plot identity had either a very small or no effect on the variation observed in the PLD by all herbivory types, external damage, and mining damage. The variation in gall damage was partially attributed to variation within the different plots (22.89%). Variation attributed to study site for all types of herbivory was 19.55%, external damage was 19.17%, mining was 2.98%, and for gall damage it was 4.98% (**Table 3.3, Table 3.4**), suggesting that most variation occurred between plots within a site, rather than between individual plants within a plot.

Table 3.3 Factors that affect the observed variation in herbivory on *Betula nana* for total herbivory and external herbivory (linear mixed effects model results). Grouped by a. all herbivory types combined, and b. external damage (chewed and skeletonized). Data is based on 55 plots in 11 study sites (kept as random effects). Random effects are presented as standard deviations and % refers to the percentage of residual variance assigned to plot and site. The fixed effect of temperature refers to the mean July temperature from 1990-2015, and precipitation refers to the mean July precipitation from 1990-2015.

Explanatory variables	Percent leaves damaged		Percent leaf area damaged		Average area damaged per damaged leaf	
Fixed effects	Estimate	95% CI	Estimate	95% CI	Estimate	95% CI
a. All herbivores						
Intercept	**-11.237**	**-16.188, -6.286**	-8.662	-18.190, 0.866	-7.616	-20.039, 4.808
Latitude	**0.129**	**0.011, 0.248**	0.087	-0.135, 0.309	0.189	-0.114, 0.493
Temperature	**0.316**	**0.164, 0.468**	0.210	-0.077, 0.497	0.129	-0.259, 0.517
Precipitation	**0.018**	**0.008, 0.028**	0.016	-0.003, 0.035	0.019	-0.006, 0.045
Collection date	-0.007	-0.046, 0.031	0.002	-0.070, 0.073	-0.023	-0.122, 0.076
Random effects	*SD*		*SD*		*SD*	
Plot	1.58E-08	*(n=55, 0.00%)*	0.301	*(n=55, 12.46%)*	0.000	*(n=55, 0.00%)*
Site	0.268	*(n=11, 19.55%)*	0.541	*(n=11, 40.32%)*	0.637	*(n=11, 12.90%)*
Residual	0.544		0.585		1.655	
b. External						
Intercept	**-11.246**	**-16.250, -6.242**	-8.613	-18.184, 0.958	-7.817	-19.646, 4.012
Latitude	0.119	-0.001, 0.239	0.079	-0.145, 0.303	0.149	-0.141, 0.439
Temperature	**0.314**	**0.161, 0.468**	0.208	-0.080, 0.496	0.136	-0.234, 0.507
Precipitation	**0.018**	**0.008, 0.028**	0.016	-0.003, 0.035	0.022	-0.003, 0.046
Collection date	-0.004	-0.043, 0.035	0.004	-0.068, 0.076	-0.011	-0.106, 0.084
Random effects	*SD*		*SD*		*SD*	
Plot	1.11E-07	*(n=55, 0.00%)*	0.317	*(n=55, 13.77%)*	0.000	*(n=55, 0.00%)*
Site	0.270	*(n=11, 19.17%)*	0.542	*(n=11, 40.44%)*	0.596	*(n=11, 11.52%)*
Residual	0.555		0.577		1.651	

PLAD and APAD were not influenced by any of the predictor variables for any of the herbivory categories (**Table 3.3**). The proportion of the variation in PLAD for all herbivory attributed to study site was 40.32% and for plot it was 12.46%. For external damage the proportion of the variation in PLAD was 40.44% attributed to site and 13.77% attributed to plot. For APAD, 12.90% of the total herbivory variation and 11.52% of the external damage variation occurred within the site, but none of the variation for total herbivory or external damage was associated to plots (**Table 3.3**).

Table 3.4 Factors that affect the observed variation in herbivory on *Betula nana* for mine and gall damage (linear mixed effects model results). Grouped by a. mine damage, and b. gall damage. Because of their low prevalence, for mine and gall damage only the percent leaves damaged was analysed. Data is based on 55 plots in 11 study sites (kept as random effects). Random effects are presented as standard deviations and % refers to the percentage of residual variance assigned to plot and site. The fixed effect of temperature refers to the mean July temperature from 1990-2015, and precipitation refers to the mean July precipitation from 1990-2015.

Explanatory variables	Percent leaves damaged			
Fixed effects	Estimate	95% CI	Estimate	95% CI
a. Mine			**b. Gall**	
Intercept	-0.955	-1.966, 0.057	0.564	-0.570, 1.697
Latitude	0.013	-0.013, 0.039	**0.029**	**0.000, 0.058**
Temperature	0.020	-0.013, 0.053	0.016	-0.021, 0.053
Precipitation	0.001	-0.001, 0.003	-0.002	-0.004, 0.001
Collection date	0.001	-0.008, 0.009	**-0.010**	**-0.020, -0.001**
Random effects	*SD*		*SD*	
Plot	0.000	(*n=55, 0.00%)*	0.092	(*n=55, 22.89%)*
Site	0.037	(*n=11, 2.98%)*	0.043	(*n=11, 4.98%)*
Residual	0.210		0.164	

Empetrum nigrum

A total of 12,456 individual *Empetrum nigrum* leaves were examined (120 individuals), and 76 had some form of invertebrate herbivore damage (<0.01%). External damage (chewed and skeletonized) occurred on 75 leaves, 1 leaf had gall damage, and none presented signs of mining. Analyses were only conducted for all herbivory damage and external damage. As well, due to the small amount of damage only PLD was examined for those two categories. The average number of leaves damaged per sample was 0.62 ± 0.19%, and the average leaf area lost per sample was 0.13 ± 0.05%.

Table 3.5 Factors that affect the observed variation in herbivory (measured as percentage of leaves damaged) on *Empetrum nigrum* (linear mixed effects model results). Grouped by a. all herbivory types combined, and b. external damage (chewed and skeletonized). Mine damage and gall damage are not included due to the small number of leaves damaged in those categories. Data is based on 40 plots in 9 study sites (kept as random effects). Random effects are presented as standard deviations and % refers to the percentage of residual variance assigned to plot and site. The fixed effect of temperature refers to the mean July temperature from 1990-2015, and precipitation refers to the mean July precipitation from 1990-2015.

Explanatory variables	Percent leaves damaged			
Fixed effects	Estimate	95% CI	Estimate	95% CI
a. All herbivores			**b. External damage**	
Intercept	-1.547	-7.446, 4.351	-1.508	-7.361, 4.344
Latitude	0.127	-0.179, 0.432	0.129	-0.174, 0.432
Temperature	0.050	-0.132, 0.233	0.054	-0.136, 0.235
Precipitation	-0.010	-0.028, 0.009	-0.010	-0.029, 0.008
Collection date	-0.035	-0.131, 0.062	-0.036	-0.131, 0.060
Random effects	*SD*		*SD*	
Plot	9.36E-07	(*n=40, 0.00%)*	4.93E-07	(*n=40, 0.00%)*
Site	0.322	(*n=9, 4.01%)*	0.320	(*n=9, 29.78%)*
Residual	0.498		0.491	

None of the predictor variables (mean July temperature (1990-2015), mean July precipitation (1990-2015), latitude, and collection date) had any effect on the variation in PLD for *E. nigrum*. Variation associated with plot was either zero or near zero for all categories. The variation in all herbivory types within the sites was 4.01% for PLD, and 29.78% for defoliation (**Table 3.5**).

Vaccinium vitis-idaea

A total of 6,935 individual *Vaccinium vitis-idaea* leaves were examined (69 individuals), and 1,181 had some form of invertebrate herbivore damage (17.03%). External damage (chewed and skeletonized) occurred on 408 leaves (5.88%), mining damage occurred on 66 leaves (0.95%), and 720 leaves (10.38%) had gall damage. The average number of leaves damaged per sample was 16.97 ± 1.52%, and the average leaf area lost per sample was 1.46 ± 0.27%.

The predictor variables (mean July temperature, mean July precipitation, latitude, and collection date) had no influence on the variation in total herbivory and external damage. A large portion of the variation in total herbivory was attributed to the differences within sites (i.e. between plots): 88.13% for PLD, 82.06% for PLAD, and 61.64% for APAD. Similarly, for external damage 65.01% was attributed to differences within sites for PLD, 64.36% for PLAD, and 66.17% for APAD (**Table 3.6**).

Mining damage was found to be positively influenced by a later collection date and negatively influenced by increasing latitude for PLD, PLAD, and APAD. Only a small proportion of the variation in PLD, PLAD, and APAD was attributed to differences between sites (12.49%, 14.70%, and 18.59% respectively), and even less was attributed differences within plots (**Table 3.6**).

The PLD and PLAD for gall damage were not influenced by any of the predictors. Variation in PLD was 67.46% attributed to differences within sites, and 3.40% to differences within plots. The variation in PLAD was 22.93% attributed to within-site differences and 7.72% attributed to within-plot differences. Gall APAD was found to be positively influenced by latitude with none of the variation associated with either plot or site (**Table 3.6**).

Table 3.6 Factors that affect the observed variation in herbivory on *Vaccinium vitis-idaea* (linear mixed effects model results). Grouped by a. all herbivory types combined, b. external damage (chewed and skeletonized), c. mine damage, and d. gall damage. Data is based on 25 plots in 6 study sites (as random effects). Random effects are presented as standard deviations and % refers to the percentage of residual variance assigned to plot and site. The fixed effect of temperature refers to the mean July temperature from 1990-2015, and precipitation refers to the mean July precipitation from 1990-2015.

Explanatory variables	Percent leaves damaged		Percent leaf area damaged		Average area damaged per damaged leaf	
Fixed effects	Estimate	95% CI	Estimate	95% CI	Estimate	95% CI
a. All herbivores						
Intercept	-11.020	-54.568, 32.528	13.207	-41.616, 68.030	20.958	-3.652, 45.568
Latitude	0.296	-0.538, 1.130	-0.243	-1.348, 0.862	-0.392	-0.924, 0.140
Temperature	0.068	-0.196, 0.331	0.005	-0.327, 0.337	-0.032	-0.183, 0.120
Precipitation	0.005	-0.024, 0.034	-0.004	-0.040, 0.033	-0.005	-0.022, 0.012
Collection date	-0.043	-0.154, 0.069	0.016	-0.139, 0.171	0.034	-0.045, 0.113

Random effects	*SD*		*SD*		*SD*	
Plot	0.115	(*n=25, 2.71%)*	0.1766	(*n=25, 4.20%)*	0.109	(*n=25, 7.58%)*
Site	0.657	(*n=6, 88.13%)*	0.7807	(*n=6, 82.06%)*	0.312	(*n=6, 61.64%)*
Residual	0.212		0.3195		0.220	
b. External						
Intercept	43.119	-18.313, 104.551	84.052	-13.793, 181.898	634.753	-107.653, 1377.159
Latitude	-0.848	-2.139, 0.444	-1.786	-3.846, 0.275	-13.618	-29.443, 2.206
Temperature	-0.028	-0.407, 0.351	-0.138	-0.742, 0.466	-1.346	-5.896, 3.203
Precipitation	-0.013	-0.054, 0.028	-0.030	-0.096, 0.036	-0.282	-0.782, 0.217
Collection date	0.076	-0.112, 0.264	0.189	-0.111, 0.489	1.588	-0.738, 3.914
Random effects	*SD*		*SD*		*SD*	
Plot	1.21E-06	(*n=25, 0.00%)*	1.73E-06	(*n=25, 0.00%)*	2.714	(*n=25, 5.19%)*
Site	0.822	(*n=6, 65.01%)*	1.304	(*n=6, 64.36%)*	9.689	(*n=6, 66.17%)*
Residual	0.603		0.971		6.374	
c. Mine						
Intercept	14.578	-0.329, 29.485	21.69	-3.792, 47.172	20.286	-2.630, 43.201
Latitude	**-0.513**	**-0.846, -0.179**	**-0.782**	**-0.352, -0.211**	**-0.649**	**-1.162, -0.136**
Temperature	-0.097	-0.225, 0.031	-0.175	-0.392, 0.042	-0.142	-0.335, 0.051
Precipitation	-0.006	-0.016, 0.004	-0.005	-0.022, 0.012	-0.004	-0.019, 0.012
Collection date	**0.099**	**0.050, 0.148**	**0.147**	**0.063, 0.231**	**0.119**	**0.043, 0.194**
Random effects	*SD*		*SD*		*SD*	
Plot	0.199	(*n=25, 12.49%)*	0.363	(*n=25, 14.70%)*	0.358	(*n=25, 18.59%)*
Site	1.05E-07	(*n=6, 0.00%)*	0.000	(*n=6, 0.00%)*	6.29E-08	(*n=6, 0.00%)*
Residual	0.527		0.876		0.749	
d. Gall						
Intercept	-194.265	-756.937, 368.407	-3.990	-11.782, 3.803	**-12.120**	**-22.042, -2.197**
Latitude	2.996	-8.896, 14.887	0.039	-0.136, 0.215	**0.263**	**0.041, 0.485**
Temperature	0.508	-2.941, 3.957	0.008	-0.045, 0.060	0.050	-0.040, 0.140
Precipitation	-0.029	-0.406, 0.349	0.000	-0.005, 0.005	0.004	-0.003, 0.011
Collection date	-0.028	-1.765, 1.709	0.006	-0.020, 0.033	-0.025	-0.058, 0.007
Random effects	*SD*		*SD*		*SD*	
Plot	1.675	(*n=25, 3.40%)*	0.048	(*n=25, 7.72%)*	0.000	(*n=25, 0.00%)*
Site	7.459	(*n=6, 67.46%)*	0.082	(*n=6, 22.93%)*	0.000	(*n=6, 0.00%)*
Residual	4.902		0.143		0.416	

Salix polaris and Salix herbacea

A total of 6,730 individual *Salix* leaves were examined (66 individuals), and 1,099 had some form of invertebrate herbivore damage (16.33%). External damage (chewed and skeletonized) occurred on 1,047 leaves (15.56%); mining damage occurred on 15 leaves

(0.22%), and 37 leaves (0.55%) had gall damage. Since so few leaves had mining or gall damage only PLD was calculated for these types of damage. The average number of leaves damaged per sample was 16.16 ± 2.62%, and the average leaf area lost per sample was 2.00 ± 0.39%.

The correlation of the predictors for this subset of study sites showed that the four predictor variables had fairly high correlation coefficients as well as high VIFs, therefore all predictors other than long-term mean July temperature were dropped. Temperature was retained due to the strong relationship reported between temperature and invertebrate herbivory (Bale et al. 2002, Danks 2004).

Temperature did not influence PLD for total herbivory, but PLAD and APAD were both positively influenced by temperature. External damage PLD, PLAD, and APAD were all positively affected by temperature (**Table 3.7**), while mining and gall PLD, were both unaffected by temperature (**Table 3.8**).

The variation in PLD and the variation in PLAD for total herbivory were both highly attributed to the differences within sites (71.90% and 60.13%, respectively). Similarly, external damage variation was also highly attributed to differences within sites for both PLD and PLAD (74.52%, and 61.80%, respectively; **Table 3.7**). Variation in mining damage was almost entirely attributed to the local differences within study sites for PLD (81.95%; **Table 3.8**).

Table 3.7 Factors that affect the observed variation in herbivory on *Salix polaris and Salix herbacea* combined for total and external herbivory (linear mixed effects model results). Grouped by a. all herbivory types combined, and b. external damage (chewed and skeletonized). Data is based on 22 plots in 6 study sites (kept as random effects). Random effects are presented as standard deviations and % refers to the percentage of residual variance assigned to plot and site. In the models for Salix temperature was included as the only predictor variable due to correlation with other predictor variables (latitude and precipitation). The fixed effect of temperature refers to the mean July temperature from 1990-2015.

Explanatory variables	Percent leaves damaged		Percent leaf area damaged		Average area damaged per damaged leaf	
Fixed effects	Estimate	95% CI	Estimate	95% CI	Estimate	95% CI
a. All herbivores						
Intercept	-0.186	-3.839, 3.468	-0.586	-1.911, 0.740	0.217	-0.434, 0.869
Temperature	0.507	-0.008, 1.021	**0.234**	**0.047, 0.421**	**0.348**	**0.257, 0.440**
Random effects	*SD*		*SD*		*SD*	
Plot	0.625	*(n=22, 9.87%)*	0.344	*(n=22, 20.21%)*	0.000	*(n=22, 0.00%)*
Site	1.687	*(n=6, 71.90%)*	0.757	*(n=6, 60.13%)*	0.000	*(n=6, 0.00%)*
Residual	0.850		0.339		1.087	
b. External						
Intercept	-1.046	-4.819, 2.727	-0.632	-2.002, 0.739	0.045	-1.641, 0.731
Temperature	**0.580**	**0.048, 1.111**	**0.234**	**0.041, 0.427**	**0.370**	**0.274, 0.466**
Random effects	*SD*		*SD*		*SD*	
Plot	0.615	*(n=22, 9.22%)*	0.344	*(n=22, 19.27%)*	0.000	*(n=22, 0.00%)*
Site	1.750	*(n=6, 74.52%)*	0.615	*(n=6, 61.80%)*	0.000	*(n=6, 0.00%)*
Residual	0.817		0.340		1.145	

Table 3.8 Factors that affect the observed variation in herbivory on *Salix polaris and Salix herbacea* combined for mine and gall damage (linear mixed effects model results). Grouped by a. mine damage, and b. gall damage. Because of their low prevalence, for mine and gall damage only the percent leaves damaged was analysed. Data is based on 22 plots in 6 study sites (kept as random effects). Random effects are presented as standard deviations and % refers to the percentage of residual variance assigned to plot and site. In the models for *Salix* temperature was included as the only predictor variable due to correlation with other predictor variables (latitude and precipitation). The fixed effect of temperature refers to the mean July temperature from 1990-2015.

Explanatory variables	Percent leaves damaged			
Fixed effects	Estimate	95% CI	Estimate	95% CI
a. Mine			**b. Gall**	
Intercept	0.199	-0.976, 1.375	0.361	-0.677, 1.399
Temperature	0.056	-0.109, 0.222	-0.132	-0.278, 0.015
Random effects	*SD*		*SD*	
Plot	0.000	*(n=22, 0.00%)*	0.000	*(n=22, 0.00%)*
Site	0.557	*(n=6, 81.95%)*	0.481	*(n=6, 61.38%)*
Residual	0.261		0.381	

Community-level herbivory

On average, the community weighted biomass lost to herbivory varied from 0% (Mull) to 6.73% (Murmansk; **Table 3.9**). The variation in the community weighted biomass lost to invertebrate herbivores could not be attributed to any of the predictor variables (collection date, temperature, or precipitation) but 87.71% of the variation was attributed to differences at the study site level (**Table 3.10**).

Table 3.9 Community weighted biomass lost to invertebrate herbivory measured as the percent of the total biomass of the focal plant species. Hol has been split in two (E: no sheep present; F: sheep present) for this table but was combined as one site in analysis.

Site	CWB$_{\text{Plot1}}$ (%)	CWB$_{\text{Plot2}}$ (%)	CWB$_{\text{Plot3}}$ (%)	CWB$_{\text{Plot4}}$ (%)	CWB$_{\text{Plot5}}$ (%)	CWB$_{\text{Avg}}$ (%)
Burntpoint Creek	0.11	0.14	0.04	0.11		0.10
Bylot Island	0.10	0.03	0.09	0.14	0.14	0.10
Pika Camp	1.39	3.00	2.28	1.38	1.34	1.88
Ailigas	0.87	0.18	0.42	0.86	0.11	0.49
Njallavaara	1.34	0.63	0.34	0.44	0.63	0.68
Audkuluheidi	0.45	0.35	0.09	0.60	0.33	0.36
Fjallabak	2.37	2.86	2.83	2.33	0.75	2.23
Skálpanes	9.23	2.73	3.88	3.31	2.67	4.36
Theistareykir	0.16	0.07	0.09	0.01	0.04	0.07
Hol E	0.96	1.08	0.78	2.63	0.12	0.86*
Hol F	0.66	0.51	0.32	0.71		
Bogstranda	0.07	0.03	0.02	0.05	0.01	0.04
Kaffiøyra	0.31	0.01	0.20	0.05	0.04	0.12
Kikutodden	1.55	1.17	0.45			1.05
Erkuta	1.44	2.19	3.50	2.88	4.14	2.83
Murmansk	7.18	4.66	4.55	4.49	12.78	6.73
Latnjajaure	0.64					0.64
Padjelanta	0.32	0.34	0.49	1.12	0.64	0.58
Val Bercla	0.13	0.48	0.41	0.17	1.14	0.47
Mull	0.00					0.00
Barrow	0.39	0.05	0.03	1.41	0.23	0.42
Toolik Lake	0.49	0.24	0.27	0.26	0.21	0.29

*average for all 9 Hol plots

Table 3.10 Factors that affect the observed variation in the community weighted biomass (linear mixed effects model results). Data come from 21 study sites (kept as a random effect). The random effect is presented as the standard deviation and % refers to the percentage of residual variance assigned to site. The fixed effect of temperature refers to the mean July temperature from 1990-2015, and precipitation refers to the mean July precipitation from 1990-2015.

Explanatory variables	CWB (% lost)	
Fixed effects	Estimate	95% CI
Temperature	-0.005	-0.151, 0.141
Precipitation	-0.002	-0.013, 0.009
Collection Date	0.013	-0.018, 0.044
Random effects	*SD*	
Site	1.009	(n=20, 87.71%)
Residual	0.378	

Discussion

The level of variation in background invertebrate herbivory in the tundra at both the species level and the community level was assessed to determine if these patterns can be attributed to climatic drivers, latitude, or collection date. Across the 22 study sites included in this study some level of invertebrate herbivory was detected at all but one site (Mull, Scotland), indicating that invertebrate herbivory is widespread in the tundra biome. However, the intensity of herbivory was generally low. At the community level, the percent of foliar biomass lost to invertebrates (excluding the site where no invertebrate herbivory was detected) ranged from 0.04% (Bogstranda, in Svalbard) to 6.73% (Murmansk, Russia; **Table 3.9**). Compared to the 2-15% lost to background invertebrate herbivory in the boreal forest (Zvereva et al. 2012), these numbers are relatively low. At the species level, the average number of leaves damaged ranged between 0.62 ± 0.19% for the unpalatable *E. nigrum* to 16.97 ± 1.52% for the more palatable *V. vitis-idaea*. These values show that while invertebrate herbivory can be observed consistently across tundra sites, the intensity of herbivory is low even for palatable plant species. The results show that at the community level, the variation in herbivory is not attributed to the climatic variables examined, nor to latitude. Herbivory at the plant species level was found to respond to temperature, precipitation, latitude, and collection date differently depending on the species and type of herbivory. In general temperature, when it had an effect, was always positive and the strongest of the predictors.

Species-level herbivory

A large proportion of the signs of invertebrate herbivory were attributed to external leaf feeders for all of the target species. External damage contributed to 82.07 ± 15.53% of the damaged leaves, while mine damage and gall damage contributed 1.92 ± 1.26% and 16.54 ± 14.82%, respectively. This higher value for external herbivory is supported by other studies that found comparatively low occurrence of galls and leaf mines in the tundra (Kozlov et al. 2015b, Barrio et al. 2017). Similarly, the fossil record reports that leaf miners and leaf gallers have historically existed in lower numbers than leaf chewers (Currano et al. 2008).

Betula nana

At sites with warmer July temperatures and higher July precipitation there were higher percentages of leaves damaged by total herbivory and by external herbivory. Warmer temperatures can lead to lower quality leaf tissue by decreasing the availability of nitrogen in the leaf tissue (Tolvanen and Henry 2001), while at the same time warmer temperatures increase the activity of invertebrate herbivores (Danks 2004, Lemoine et al. 2013). This results in higher energy demands for the herbivores and therefore an increase in food consumption (Lemoine et al. 2013, 2014). The resulting increase in the number of *B. nana* leaves damaged by herbivores is therefore to be expected at warmer study sites. A similar pattern of increased invertebrate herbivory with increased temperature and precipitation was found by Kozlov (2008) and Barrio et al. (2017).

The percentage of leaves damaged by galls increased at higher latitudes. This could be attributed to the low number of galls detected. Galls were only found in 5 of the 165 samples, and of those samples four were from the same site (three from the same plot), and the last sample from a different site that had the same latitude.

The percent of leaves damaged by galls was negatively affected by the time of collection. Sites that collected leaves later in the season had fewer leaves with gall damage than those collected earlier in the summer. This could be attributed to gall damage causing early leaf abscission (Faeth et al. 1981, Zvereva and Kozlov 2014) resulting in the loss of the leaves that were damaged by galling invertebrates.

Empetrum nigrum

Herbivory on *E. nigrum* was minimal, as expected given the low palatability of this species to herbivores (MacLean Jr. and Jensen 1985). Most of the herbivory reported on *E. nigrum* originates from Murmansk, which at the time of data collection had a reportedly high number of sawfly larvae (Kozlov, *personal communication*). The resulting herbivory on *E. nigrum* is likely due to spillover from herbivores that had exhausted the preferred food source (Jepsen et al. 2013, Karlsen et al. 2013).

Vaccinium vitis-idaea

Of the four target species *V. vitis-idaea* was the only species in which the most common damage type was not external damage (34.55% of damaged leaves): the most common type of damage were galls (60.97% of damaged leaves). *V. vitis-idaea* was also the species with the highest number of mine damaged leaves with 0.95% of the total leaves having mines, compared to 0% (*E. nigrum*), 0.10% (*B. nana*), and 0.22% (*Salix*). All the measurements of mining damage (PLD, PLAD, and APAD) were positively affected by the collection date. Sites at which *V. vitis-idaea* leaves were collected later in the growing season had more mining damage due to the accumulation of damage over time. Some studies have shown that herbivory damage by leaf miners can lead to early leaf abscission (Pritchard and James 1984, Zvereva and Kozlov 2014); this would result in fewer damaged leaves found later in the season, opposite to our results. Arguments have been made as to the exact affect that leaf miners have on leaf abscission, and whether they may actually delay leaf abscission (Pritchard and James 1984). Since leaf miners reside inside the leaf, early abscission would lead to high mortality of these herbivores (Faeth et al. 1981, Oishi and Sato 2007), and therefore it would make sense that they could have mechanisms to avoid premature leaf abscission (Oishi and Sato 2007). Some species of leaf miners have been found to induce the formation of cytokinins; the chemical that prevents leaf senescence (Pritchard and James 1984, Oishi and Sato 2007). Oishi and Sato (2007) found that leaves containing living larvae of the lepidopteran leafminer, *Coptotriche japoniella,* did not abscise prematurely, allowing the larvae to finish feeding. Based on these findings it is possible that the species of leaf miners feeding on the *V. vitis-idaea* leaves may be suppressing leaf senescence allowing for the accumulation of mine damage.

Latitude had a negative effect on all the measurements of mining damage on *V. vitis-idaea*. Sites at higher latitudes had less mine damage than those at lower latitudes, even with there being a relatively small latitudinal range (67.31° - 71.30°). This is consistent with the Latitudinal Herbivory Hypothesis, as well as with results from Kozlov et al. (2013), where they found lower diversity and abundance of leaf mining species at higher latitudes. While their study focused on *B. pendula* and *B. pubescens,* the same trend can be seen here for *V. vitis-idaea*.

Salix polaris and Salix herbacea

At sites with warmer July temperatures there were higher percentages of total leaf tissue lost to herbivory, as well as a larger average percent leaf area eaten. External leaf herbivory was similarly influenced by temperature with regards to all three measurements; more leaves, more leaf area, and a higher average leaf area were consumed by externally feeding herbivores at sites with warmer temperatures. The leaves that were being eaten were being consumed to a greater extent before the herbivore switched to a different leaf in the warmer sites. This is possibly also related to the fact that these plant species rely on fast compensatory growth rather than on chemical defense in response to herbivory (see **Chapter 2**). As mentioned above, warmer temperatures result in low quality leaf tissue as well as increased invertebrate activity (Lemoine et al. 2013, 2014). Therefore, at higher temperatures more leaf tissue is consumed, especially for the leaves with high nutrient levels, such as *Salix* (Beerling 1998).

Community level variation

When examining the variation in background invertebrate herbivory at the community level none of the selected predictor variables explained the variation. At the community level, the insensitivity to our predictors could be because invertebrate herbivory may be more species specific and in the process of summing the different species we may be ignoring some important differences in leaf palatability. The site with the highest percent biomass lost to invertebrate herbivory was Murmansk (6.73%), with the second highest site being Skálpanes, in Iceland (4.36%). The lowest biomass lost to invertebrates was 0% at Mull, and the second lowest was in Bogstranda (0.04%). Two of the three sites in Svalbard reported low levels of CWB (0.04% and 0.12%), this can be attributed to the archipelago having a low number of invertebrate herbivore species. Other high latitude islands may have low levels of invertebrate herbivory due to a similar lack of herbivores, but knowledge on the species present in these sites is minimal. To our knowledge no other study has looked at the community biomass lost to invertebrate herbivores in the tundra, as the few studies available have focused on one (Barrio et al. 2017) or a few plant species (Kozlov et al. 2015).

At what scale does invertebrate herbivory vary?

While some of the variation in species level herbivory was explained by temperature, precipitation, collection date, and latitude, there was a large portion of the variation that was associated with local site characteristics (i.e. variation between plots within a site), while very little variation was associated with the plot level (i.e. between individuals plants within a plot). This trend was the strongest in *V. vitis-idaea* and *Salix*, with most measurements of herbivory having >60% of the variation associated with site. As well, the variation in CWB was not attributed to differences in temperature, precipitation, or collection date at all; 87.18% of the variation was associated with site differences. Therefore, local site characteristics that drive differences between plots and were not examined in the present study may have a stronger effect on the variation in background invertebrate herbivory. Potential factors that may be driving the levels of herbivory include snow cover (Torp et al. 2010a, 2010b), soil nutrients (Semenchuk et al. 2015), and local plant and invertebrate species composition (Strathdee and Bale 1998, Bale et al. 2002, Kozlov et al. 2015b).

Increased snow cover during the winter, either in the form of more snow fall or longer snow cover, has been linked to increases in leaf nitrogen content in *B. nana,* as well as a subsequent increase in invertebrate herbivory during the growing season (Torp et al. 2010a, 2010b). Similarly, increased snow cover resulted in higher nitrogen content in *Salix polaris* leaves (Semenchuk et al. 2015). In snow manipulation experiments Torp et al. (2010a, 2010b) reported increased levels of invertebrate herbivory in the increased snow treatments. Snow cover is an important abiotic factor in tundra ecosystems that can vary substantially on a local scale due to variations in topography (hollows with deep snow vs. windswept areas with little snow) (Torp et al. 2010a, 2010b). Accounting for the variation in snow cover would require local measurements of snow cover and snow-off dates to be added to this analysis.

Another aspect that may be important in determining the level of invertebrate herbivory is plant apparency. According to the idea of plant apparency, plants that are more visible (or apparent) to herbivores are more likely to be consumed (Zverev et al. 2017). Therefore, if a site has patchy vegetation cover those patches stand out and will have higher consumption than a site with a consistent layer of vegetation. Similarly, taller shrubs would stand out from low lying vegetation. In their study, Zverev et al. (2017) found that the patterns in invertebrate herbivory that they reported could be explained best by differences in plant apparency.

In relation to leaf quality, the nutrient levels in the soil could also have a potential effect on herbivory rates (Semenchuk et al. 2015). Plants grown in high nutrient soils, especially nitrogen and phosphorous, can potentially have leaves with high nutrient content resulting in higher palatability (Semenchuk et al. 2015). Further, invertebrate themselves can represent a potential source of nutrient inputs (Hunter 2001, Metcalfe et al. 2016). The input of excessive frass and invertebrate bodies occurring during an outbreak has been shown to increase the level, and availability of nutrients in the soil (Kaukonen et al. 2013, Metcalfe et al. 2016).

Lastly, one of the potential drivers of the variation in herbivory levels could be the species composition of the invertebrate assemblages. We assume based on past research that increased temperature would result in higher numbers of invertebrates (Hodkinson and Bird 1998, Bale et al. 2002, Callaghan et al. 2004, Wolf et al. 2008, Birkemoe et al. 2016), but there is considerable variation depending on the invertebrate species being considered. Depending on their life history, an invertebrate species may respond to changes in climate in different ways (Strathdee and Bale 1998, Bale et al. 2002, Kozlov et al. 2015b). Cold adapted species may show negative trends in survivorship, abundance, and activity with increased temperatures (Bale et al. 2002, Amarasekare and Sifuentes 2012, Barrio et al. 2016).

Conclusion

The variation in background invertebrate herbivory in the tundra biome, at both the species and community level, was related to climatic variables, but most variation was associated with local site characteristics. Potential site characteristics that could have an effect on background herbivory include snow cover, plant apparency, soil nutrient levels, and species composition. Research on background invertebrate herbivory will need to focus on elucidating the factors driving the variation by including some measurement of these suggested factors. Once the sources of variation are included we can incorporate temporal studies to examine how background invertebrate herbivory will vary across years and under a changing climate, and how this variation may subsequently affect the overall plant community.

Chapter 4: Summary: Assessing the Common Background Herbivory Protocol and Next Steps

Overview of main results

The main objective of this project was to determine the extent and level of variation in background invertebrate herbivory in the tundra and identify the potential drivers of that variation. Our results show that at the species level the patterns in invertebrate herbivory are driven by different factors depending on the plant species and the herbivore feeding type, while the drivers of herbivory at a community level are still unknown. Overall, the predictor variables of long term mean July temperature and precipitation, collection date, and latitude did have some effect on the patterns in invertebrate herbivory for *Betula nana, Salix herbacea/polaris,* and *Vaccinium vitis-idaea* (**see Chapter 3**). With regards to *Empetrum nigrum* the amount of herbivory was so low that there was little variation between the sites at all. An interesting result of this study showed that some of the main drivers of background invertebrate herbivory, especially at the community level, remain to be determined. At both the species level and at the community level much of the variation in herbivory levels could be attributed to site differences that were not related to the selected predictor variables.

Common protocol alterations

The assessment of the herbivory patterns in the tundra involved the use of different habitat types spread across the tundra biome. This large-scale study required a consistent methodology such that data collected in one area by one researcher could be compared to data collected at another area by a different researcher. Defining these consistent methodologies resulted in the design of a common protocol (**Appendix 1**). Based on the results from this study modifications to the original protocol (**Appendix 1**) will be made in order to further develop a common protocol specific to measuring background invertebrate herbivory in the tundra.

Potential drivers that were not included in the original methodology that could be incorporated into future iterations include measuring the timing of snow off and plant apparency. Some studies have shown that levels of herbivory in the tundra can be related to the extent and duration of snow cover (Berg et al. 2008, Torp et al. 2010a, 2010b). This has been related to an increase in the nitrogen content in leaves following increases in winter snow cover (Torp et al. 2010a, 2010b, Semenchuk et al. 2015). Due to differences in topography, such as hollows vs. windswept fields, and species composition (e.g. the presence of taller shrubs leading to snow build-up) the level of snow cover can vary depending on the local conditions (Torp et al. 2010a, 2010b, Myers-Smith et al. 2011). Variation in herbivory could also be attributed to the level of plant apparency. The extent of cover (e.g. patches of vegetation stand out) and growth forms of the plant species that make up the site vegetation (e.g. taller shrubs stand out and are thus more apparent to herbivores) can determine how easily invertebrate herbivores may be able to locate a food source. Zverev et al. (2017) concluded that the patterns in invertebrate herbivory they observed supported plant apparency as a mechanism driving herbivore damage. Including a measurement of plant apparency and snow cover in future iterations of the protocol could help identify how much of the site variation in herbivory is attributed to these factors.

Adding a measurement of the number of growing degree days (GDD) could lead to a more accurate indication of how climate affects invertebrate herbivory. The GDD would allow for a more fine scale measurement of the changes in temperature throughout the growing season, yet it would require more time and energy to collect site specific data.

When examining the variation in herbivory for the four target species, most variation was associated to plots within a site, while almost no variation was attributed to the differences within plots. Therefore, sample collection efforts could be better applied to include more plots within a site, potentially representing more habitat types and capturing the local variation. In regards to the species that are included as focal species, some further distinction could be made in order to avoid excessively small leaved plants, such as *E. nigrum*. An argument can be made to keep this species in the protocol mainly due to its high frequency of occurrence, circumpolar distribution, and high degree of plant cover and biomass. On the other hand, the effort involved in examining the small leaves is high, with results showing no herbivory except in the case of outbreaks.

Results indicate that, at least for some species, there may be an effect of collection time on the level of herbivory quantified. The protocol did not directly look at the timing of herbivory. Only a single point in time, during the peak of vegetation growth, was examined for each study site. The mid-season timing was chosen such that an accumulated measure of herbivory could be examined. In the tundra, most herbivory occurs in the early growing season due to the higher palatability of young leaves (Kozlov and Zvereva 2014, Zverev et al. 2017). This design therefore includes early and mid season damage combined. In future studies it may be useful to examine the effects that different timing could have. The timing of defoliation, in regards to the leaf's lifespan, can also determine how detrimental the loss of its photosynthetic ability is to the plant. Young leaves are formed by expending carbohydrate reserves and will need to grow before they start to produce sugar to replace those carbohydrates (Stephens 1971). The loss of a young leaf to herbivores therefore results in a loss of carbohydrates. An old leaf that is near senescence has already contributed to the carbohydrate reserves, likely in excess of the energy used to form that leaf. Therefore the loss of an old leaf would generally have no negative effect on the plant (Crawley 1983). This was observed by Reeks and Barter (1951) when they examined the defoliation of white spruce (*Picea glanca*) and black spruce (*Picea mariana*) by spruce sawfly larvae (*Gilpinia hercyniae*), which have a preference for old needles (> 1 year). It was found that these trees can tolerate multiple years where old needles are almost entirely defoliated as long as the new needles are not consumed, although there was still a noticeable decrease in ring growth.

Data collection included some deviations from the suggested protocol. Of these deviations most were minor and could be rectified easily, but some resulted in the data being un-useable. These major deviations tended to occur regarding biomass collection. Since an estimate of the focal species biomass is needed in order to determine the community weighted biomass lost to herbivory, the estimation of biomass needs to be carried out in a consistent, comparable method. However, there were some slight problems with the methodology used for estimating biomass in this study. When collecting point-intercept data the use of only 10 pins for a 50 cm x 50 cm frame was found to be small. It is suggested that more pins (~50) be used for this frame size. This would help minimize the error surrounding properly recording the biomass for plant species that have a clumped growth habit, such as *Silene acaulis*.

When looking at the spatial distribution of the sites with high levels of community invertebrate herbivory compared to those with low levels (**Figure 4.1**), it appears that the sites with more reported herbivory occurred in Northern Europe, and the sites with almost no herbivory occurred in Canada or on islands. There could be an additional effect of site location that is not based on latitude. Including some more site location characteristics, such as the slope (mountain vs. valley), and the distance from major water bodies, could help determine if there is a pattern in invertebrate herbivory based on site geography.

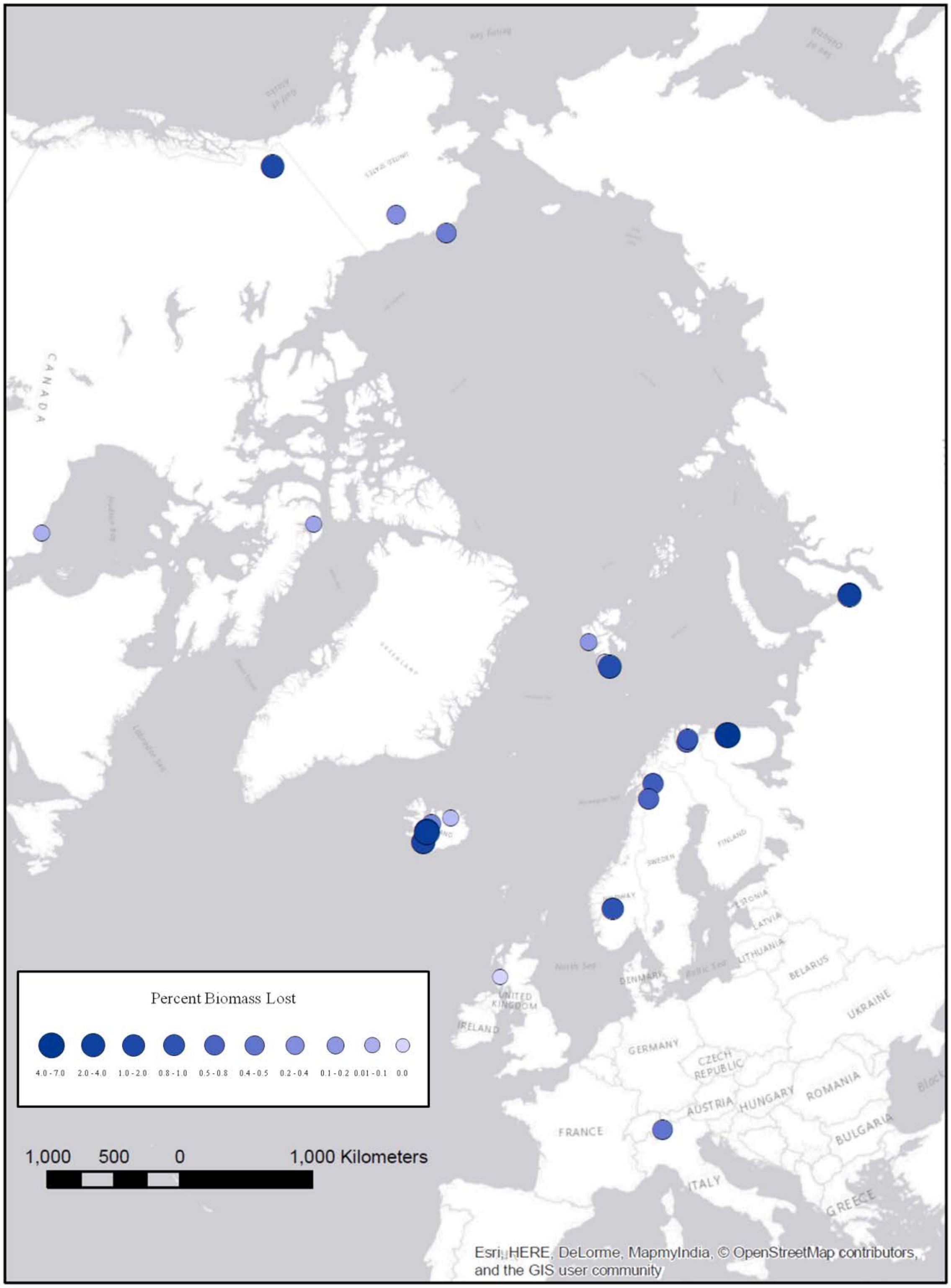

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Figure 4.1 Representation of the community weighted biomass lost to invertebrates for each site (excluding Severnaya). The larger darker circles represent sites with high CWB, and the smaller lighter circles represent sites with low CWB. Bins for each circle size and colour were determined based on natural groupings of the CWB values for each site.

Leaf damage assessment by visual inspection has been found to be a cheap and effective method of estimation, yet some variation has been found to be caused by observer differences (Johnson et al. 2016). This protocol intended to be designed such that it can be implemented by multiple researchers; therefore an assessment of potential observer effects was incorporated. Based on the assessment of different observers we can conclude that for the most part leaf analysis can be completed by multiple observers, with the stipulation that complex leaves are either all assessed by the same observer, or have a guide to common errors in assessment (**Appendix 2**).

Another aspect of the common protocol that was not included in this iteration that still needs to be examined is the temporal scale. At this stage in development determining a time frame for the frequency of data collection will be difficult since some of the main drivers of the patterns have yet to be determined. If snow cover is determined to be a huge determining factor then maybe annual measurements may be necessary due to high variation in cover year to year (Derksen et al. 2017). If plant apparency is a major driver then less frequent sampling would make sense in order to catch changes in site plant composition. Lastly, when thinking about the temporal scale of measuring background herbivory, invertebrate outbreaks need to be accounted for. Invertebrate species, such as the moths *Operophtera brumata* and *Epirrita autumnata*, show outbreak cycles of about 10 years (Jepsen et al. 2008). If outbreaks do become more frequent, as predicted (Jepsen et al. 2008, 2013, Karlsen et al. 2013), then the frequency of sampling background herbivory data should be increased such that multiple years are sampled between outbreaks.

Implications

With the current trends in climate change and the expected amplified effects in tundra ecosystems (Huntington et al. 2005, Post et al. 2009, IPCC 2013, Overland et al. 2017), understanding the responses of invertebrate herbivores allows us to predict shifts in plant-herbivore dynamics. Temperatures are projected to increase by 6-10°C in the next 100 years (IPCC 2013), and currently temperatures in the arctic are increasing at a rate two times as fast as mean global temperatures (Overland et al. 2017). While we found that increased temperatures (based on a spatial analysis comparing sites with different climate factors) has a positive effect on at least two of the focal species included in this analysis (*B. nana* and *S. herbacea/polaris*), we also found that at the community level herbivory was not affected by temperature differences. This implies that the effect of increased temperatures in the tundra may be species specific. Similar projections of increased levels of herbivory have been made by Barrio et al. (2017) for *B. nana* in the tundra.

The percent biomass lost to invertebrates at background levels was found to be relatively small (0.00-6.73%), with only 6 of the sites having more than 1% biomass loss. While these numbers are small, Zvereva et al. (2012) found that even a loss of just 2% of the leaf area for *Betula pubescens* in northern forests resulted in decreased tree growth by ~30%. Not much research has been done to examine the effects of background invertebrate herbivory on the growth of tundra shrubs, and, to our knowledge, no studies have looked at how background losses affect the overall plant community. If we assume a similar pattern to what Zvereva et al. (2012) found, we can expect that the small numbers we reported still have large impacts on the plant community. It would be interesting to look into the community level effect of the removal of such small percentages of biomass so that we can determine how much of a biological difference there is between 4% biomass loss and 0.5%.

In order to make predictions regarding changes in background invertebrate herbivory the baseline level needs to first be determined; how much damage do invertebrates cause, how much variation is there in this damage, and what drives this variation? By answering these questions, we can determine the current patterns in herbivory in order to assess how they change over time, and with increasing changes in the climate. In this study, temperature and precipitation were identified as potential drivers for the variation in species level herbivory at a biome-wide scale, as well as the timing of leaf collection and latitude. Aside from those factors much of the variation was associated with differences within sites. This was also seen at the community level, where none of the predictors explained the variation. Potential local factors that should be examined in the future include a measurement of snow cover and/or snow-off dates, and a measure of plant apparency, since both have been shown to explain some patterns in herbivory (Torp et al. 2010a, 2010b, Zverev et al. 2017). By making some of the previously mentioned changes to the background invertebrate herbivory protocol we hope to design a protocol that can allow us to determine what the main drivers in the patterns of herbivory are, and therefore allow us to predict how climate change may affect the patterns of herbivory, both spatially and temporally.

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Appendix 1: Measuring background invertebrate herbivory in the tundra

A common protocol was designed at the beginning of this project. This protocol outlined the study design and sampling procedure for the collection of leaves and biomass during the summer of 2015. This protocol was sent out through the Herbivory Network and 30 researchers responded by collecting data from 22 different circumpolar study sites. The following is the original document outlining the protocol that was sent out. Note that the names of the different spatial levels have been changed from what they are in this protocol. In the original document Study Area = Study Site and Site = Plot.

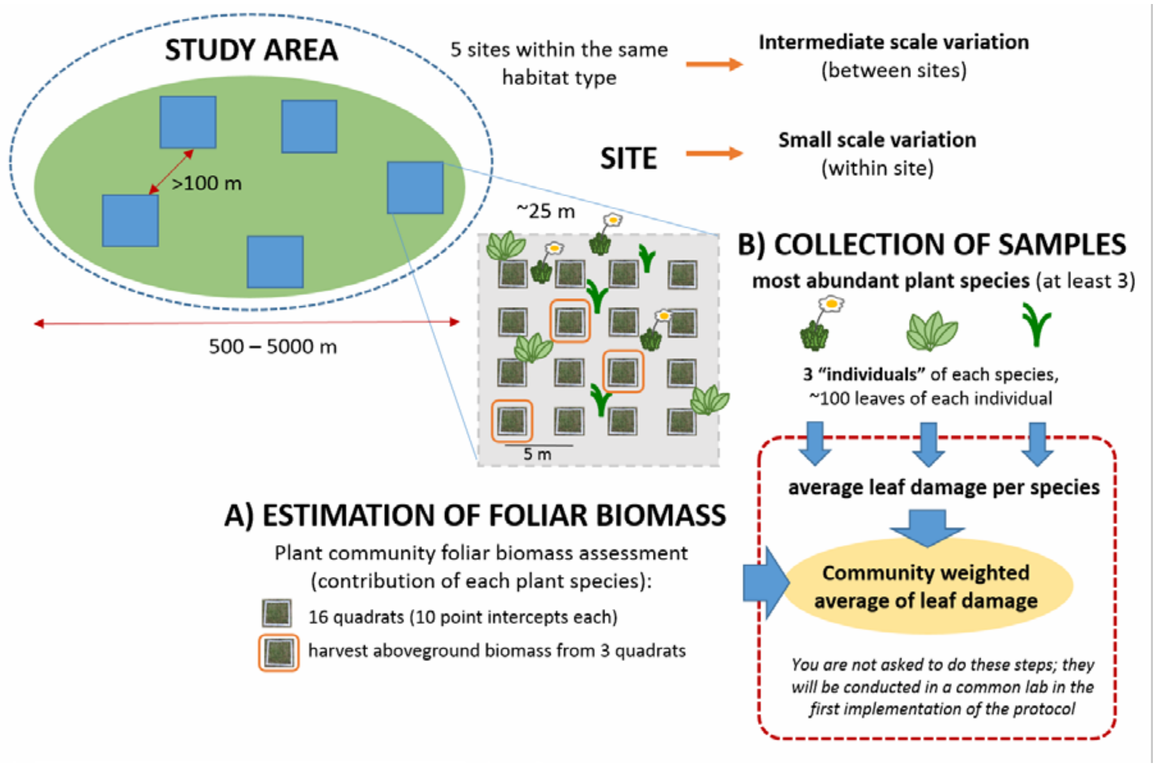
Measuring background invertebrate herbivory in the tundra

Invertebrate herbivory in tundra ecosystems has received little attention[1] and effects of foliar losses to invertebrates on tundra plants have been generally neglected[2]. Most attention has focused on extensive defoliation events during outbreaks in the forest-tundra ecotone[3], but we know little about background (non-outbreak) herbivory levels in tundra[4], despite the relevant effects it has in other systems[5].

The goal of this document is to provide guidelines for assessment of the occurrence and intensity of invertebrate herbivory at different tundra sites. In this initial assessment (summer 2015), samples will be collected from the field from a large number of tundra sites, and leaf damage will be assessed in a common lab by the same observer. This information will allow a quantitative evaluation of invertebrate herbivory, providing information to address the following questions:

- ✓ What are the average levels of background invertebrate herbivory in tundra?
- ✓ Is invertebrate herbivory similarly prevalent across tundra sites?
- ✓ Which plant species suffer most from invertebrate herbivory?
- ✓ How variable is the occurrence of invertebrate herbivory in tundra at different spatial scales?

Most studies investigating invertebrate herbivory have focused on leaf damage[6]. Signs of other types of invertebrate herbivory are not as easily recognizable (e.g. sap feeders, root herbivores) or sampling may require more intensive protocols (e.g. floral herbivory and seed predation). Although the effects of these herbivores might be as relevant as those of folivores[7,8], for a preliminary assessment of invertebrate herbivory in tundra we will focus on the impacts of herbivores that cause leaf damage.



You are not asked to do these steps; they will be conducted in a common lab in the first implementation of the protocol

Figure 1. Study design for assessing variability in invertebrate herbivory at two spatial scales, between and within sites.

Information collected in this study will contribute to the development of a more general protocol to measure invertebrate herbivory in tundra, but also represents a standalone project. All contributors will be welcomed to co-author the resulting publication. This first assessment will focus on **spatial variability** of leaf damage; temporal variability, with large annual variation in herbivory (e.g. [8,9]), will be accounted for in future implementations, by conducting annual observations over several years. For the assessment of spatial variability and to calculate sample sizes needed to capture this variation more accurately in the future, sampling in the first implementation (summer 2015) will be structured within a study area, in a minimum of 5 sites (intermediate scale variation) within a common habitat type, sampling plants of the most abundant species within each site (small scale variation; **Figure 1**).

Study areas are loosely defined here at a scale of hundreds of meters to a few kilometers (i.e. areas separated more than 5 km apart would be considered different study areas); study area is, broadly, the place where you conduct your research. Within each study area, sampling will focus on the most dominant, typical habitat type (i.e. zonal habitat, *sensu* CAVM: areas where the vegetation develops under the prevailing climate, uninfluenced by extremes of soil moisture, snow, soil chemistry, or disturbance[10]). We suggest to sample in at least **5 sites** (corresponding to the same habitat) that will be at least 100 m apart. Sites will have a size of approximately 25x25 m – please note that this size is indicative and it is intended to give an idea of the spatial scale at which the variation will be accounted for. Please write down the habitat type and **take pictures** of all 5 sites! In this first assessment of invertebrate herbivory, sites should NOT be linked to existing monitoring plots, especially because sampling will be destructive (we are asking you to collect a lot of leaves! So probably you do not want to do that in your study plots). Sites should correspond to the same habitat type. Then, in each study area, the focal species (see below) will probably be the same in all 5 sites. You are more than welcome to include additional habitats, but each habitat type should then be represented by at least 5 additional sites.

In each site, you are asked to do two things:

A) **Estimate foliar biomass at the community level.**Plant community assessments accounting for foliar biomass of each species need to be conducted for each site to allow calculation of the community-wide losses of foliage to invertebrate herbivores from the species-specific data. Plant cover will be estimated using the point intercept method[11], using 10 pins in a fixed position within a 50x50 cm frame, where all plant species that hit the pins will be recorded. For the three focal species it will be recorded if each hit corresponds to leaves or woody parts. 16 frames will be placed in a regular grid at each site, 5 m apart, to cover a 20x20 m area (**Figure 1 and overview**). From 3 of these frames aboveground biomass will be collected. These frames should ideally represent a range in abundance of the focal species (i.e., minimum, maximum and average hits) because we will then need to calibrate the number of hits to the foliar biomass of each species, so the broader the gradient we can get, the better – but if this proves to be too complicated to assess in the field, a random selection of 3 of the 16 frames will do. Live green biomass samples from each frame (it is extremely important to label uniquely the point intercept recording and the corresponding biomass collection!) will be sorted by species (at least for the three focal species) and dried in an oven (~70°C, 72 h), and packed together with the rest of the samplesfor shipping.

B) **Collect leaf samples of the most abundant species.**A number of focal **plant species** (minimum of 3) will be identified based on their relative contribution to foliar biomass in each site. The most abundant plant species (the one contributing the most foliar biomass at each site) should be selected first, followed by the second and third most abundant. The more species per plot the better community estimates we will have, but the minimum is **three**; importantly, selection of focal species should not be biased towards plants receiving higher-than-average levels of foliar damage[12]. For example, some species of arctic plants (e.g. *Salix* spp.) carry a disproportionate number of herbivorous species[13]. From each plant species in each site, samples from 3 individuals will be collected. Defining "individuals" in the tundra can be tricky, because many plants are clonal; however, plant genotypes vary in their susceptibility to insect herbivory and therefore it is important to sample whenever possible several genotypes per site, at least at this test stage. For our purposes, individual plants (genets) can be defined as plants separated at least 10 m. For plants with smaller stems (e.g. *Vaccinium vitis-idaea* and *Salix rotundifolia*) or with less leaves per individual, several stems living close together (e.g. within 1-2 m) can be aggregated to collect ~100 leaves from 3 different "aggregates". Individual plants (or aggregates) should be "blindly" selected from a distance that prevents recognising foliar damage by insects (e.g. 5-10 m)[12]; a number of individuals of each species in each site might be temporarily located using flags and then, from a distance, randomly select 3 flags and sample the corresponding individuals. Please try not to bias your sample to a specific height of the plant above the ground when you are collecting leaves (i.e. sample across all heights). Leaf damage levels in tundra are low (<1% on woody species[4]), so a relatively large sample (i.e. sufficient nr of leaves) needs to be collected for each individual. Based on a previous study reporting background herbivory levels in tundra[4], we expect that less than one leaf out of 100 might be completely eaten (although most likely we will observe minor damage in a few leaves), so we will need to collect ~100 leaves from each individual to estimate damage accurately. Leaf samples can be temporarily stored in labelled plastic bags (labels should include information on study site, patch, plant species and plant individual), and be processed for preservation back in the lab. In this way, field sampling is not as time consuming, as entire plants or branches are collected in the field.

Leaf samples should be preserved as herbarium specimens (pressed and dried, to avoid growth of mould). Newspaper used for pressing plants needs to be changed daily in the first couple of days to ensure samples are kept dry. For plants with relatively large leaves (leaf size of *Betula nana* or greater), leaves should be detached from the stems before pressing, so that leaf damage can be easily identified on flat, unfolded leaves. Species with needle-like leaves (e.g. *Empetrum nigrum*, *Loiseleuria procumbens*) tend to have early abscission of leaves after damage, and this can be assessed by scars left on twigs after leaves are dropped; for these species it is therefore important to keep leaves and stems together when pressing them.

Pressed samples will be shipped to the University of Iceland and processed in the lab by the same observer (ICB). So, up to here, it is what you are asked to do; but if you are curious on what will happen to the samples after you ship them (in the red box in **Figure 1**), please continue reading☺

In the lab, leaves will be carefully assessed for damage on both sides and through a source of light (some damage types are not so obvious to the naked eye). Each leaf will then be attributed to a damage class according to the proportion of area of leaf lamina that is consumed or damaged by insects: intact leaves, 0.01-1%, 1.01-5%, 5.01-25%, 25.01-50%, 50.01-75%, and 75.01-100%.Most foliar damage in tundra is imposed by leaf chewers, followed by gallers (the incidence of leaf miners, if any[4], is very low), and evidence from leaf damage by each group will also be recorded separately when possible. The numbers of leaves in each damage class will be recorded, and for each individual plant the following measurements will be calculated:

1. **Proportion of damaged leaves:** ratio between the number of damaged leaves and the total number of surveyed leaves (separate measurements can be calculated for defoliators, miners, gallers and all herbivores combined)
2. **Average proportion of leaf area lost to insects:** the number of leaves in each damage class is multiplied by the respective median of the damaged leaf area (0 for intact leaves, 0.5% for the damage class 0.01-1%, 3% for damage class 1.01-5%,…); the obtained values are summed across all damage classes and divided by the total number of leaves (including undamaged ones) within a sample.

From the dry weights of the biomass samples collected, we build a calibration between the number of pins recorded in the field with the point intercept method and foliar biomass, to estimate the relative contribution of each plant species to community foliar biomass. Community estimates of biomass loss can be then obtained by doing a weighted average for the foliar biomass removed by invertebrates on the dominant species in the community.

Additional information that can be collected opportunistically…

Additional information on unusual situations or ecological surprises related to invertebrate herbivory is also highly needed. Although the focus here is set on describing "typical situations" of invertebrate herbivory, opportunistic information on extremes is also highly valuable. If you happen to observe an unusually high level of plant damage, then please (a) take a photograph of the damage type and the damaging invertebrate, whenever possible; (b) preserve the damaging invertebrate in alcohol for future identification; (c) estimate the spatial extent of the excessive damage; and (d) collect leaf samples from at least 5 plant individuals both within and outside the 'exceptionally damaged' area.

Our knowledge of Arctic entomology is still relatively limited[14], so it is also very interesting to know who is actually consuming the leaves. Whenever possible, please take pictures, collect and preserve insects seen on plants, along with a damaged part of the plant, and record if the insect was actually feeding on the plant or just sitting on it. When sufficient amounts of specimens are accumulated, we will transfer them to relevant taxonomists. In particular, aphid samples from tundra are especially needed and will be processed in the first line. Knowing the feeding habits of the invertebrates that are most commonly observed to damage plants in your study areas will help develop the protocols further.

Timing and time commitment

Invertebrate herbivory data should be collected once at the end of the growing season (cumulative signs of herbivory might be better assessed later in the season, before plant senescence). No big differences between mid and late summer in insect herbivory are expected[4,9].We expect sampling to take **one day of work in the field** per study area for one person over the whole season (max up to two days). A rough estimate of time dedicated to each activity (needs to be adjusted to each site):

- Estimates of community foliar biomass: 16 point frames and collection of biomass in 3 50x50 cm frame ~60 minutes in the field per site: ~5 hr in the field
- Collection of leaves (search of individual plants of each focal species in a patch and collection of 100 leaves per plant): 1 hr per plot, ~5 hr
- Processing and preserving samples (pressing samples): ~ 3 hr for all patches, but a bit of attention in the following days

Collected samples and data

Data collected from the plant community composition assessments, using the proposed field sheets (see appendix) or your own templates, can be entered following the data entry procedure described in the appendix, or scanned copies of the field sheets can be sent to us (herbivory.network@gmail.com) at the end of the field season.

If you have any questions, please contact: icbarrio@gmail.com

Leaf samples and biomass samples should be sent to:

Isabel C Barrio
Institute of Life and Environmental Sciences
University of Iceland
Sturlugata 7, IS-101Reykjavik, Iceland

Materials

- Frame (50x50 cm) and pins – for assessing foliar biomass of plant community
- Field sheets and pencil, to record data from point intercepts
- Large paper bags – for collecting biomass samples (15 per site, 75 total)
- Plastic bags for temporary storage of leaf samples (one per individual plant (3 individuals of 3 species at each site), minimum of 45 bags total).
- Permanent marker – for labelling bags indicating site, date, plant species and collector name.
- Clippers or pruning scissors – to collect aboveground biomass, pruning scissors might be needed to collect branches of woody species
- Tape measure 25m long (for delimiting sites)
- Camera – to take pictures of the sites

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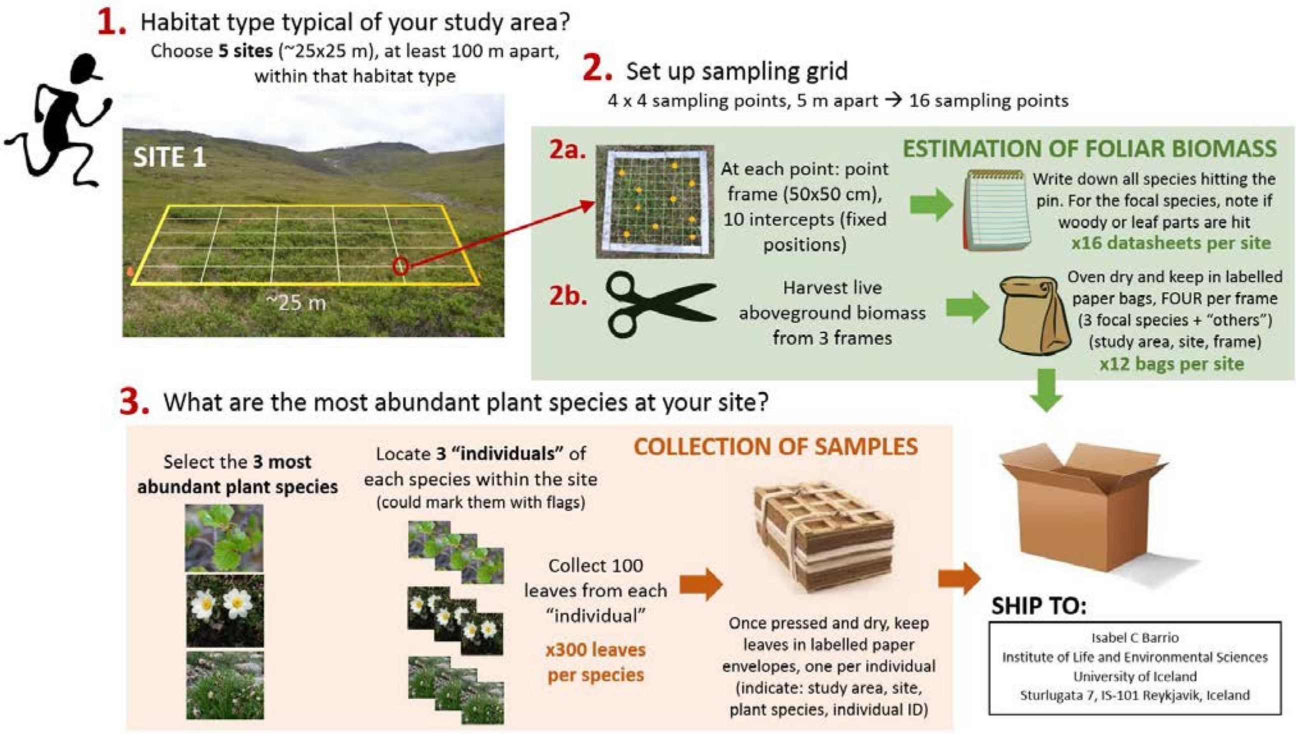
Overview of the sampling protocol



1. Habitat type typical of your study area?

Choose **5 sites** (~25x25 m), at least 100 m apart, within that habitat type

2. Set up sampling grid

4 x 4 sampling points, 5 m apart → 16 sampling points

ESTIMATION OF FOLIAR BIOMASS

2a. At each point: point frame (50x50 cm), 10 intercepts (fixed positions) → Write down all species hitting the pin. For the focal species, note if woody or leaf parts are hit **x16 datasheets per site**

2b. Harvest live aboveground biomass from 3 frames → Oven dry and keep in labelled paper bags, FOUR per frame (3 focal species + "others") (study area, site, frame) **x12 bags per site**

3. What are the most abundant plant species at your site?

COLLECTION OF SAMPLES

Select the **3 most abundant plant species**

Locate **3 "individuals"** of each species within the site (could mark them with flags)

Collect 100 leaves from each "individual" **x300 leaves per species**

Once pressed and dry, keep leaves in labelled paper envelopes, one per individual (indicate: study area, site, plant species, individual ID)

SHIP TO:

Isabel C Barrio
Institute of Life and Environmental Sciences
University of Iceland
Sturlugata 7, IS-101 Reykjavik, Iceland

Appendix 2: Cross Calibration

Introduction

The protocol for measuring invertebrate herbivory in tundra ecosystems designed by *The Herbivory Network* (**Appendix 1**) standardizes data collection in the field, but implies damage assessment by a single observer in the lab. A potential extension of this protocol would be for different observers to conduct leaf damage assessment. However, this critical step may involve biases in the visual assessment of damage, especially if observers have different experience. Here, a cross-calibration analysis is implemented to evaluate the potential effects of differing observers in the assessment of leaf damage. The results of this cross-calibration will determine if multiple observers can assess leaf damage in this protocol.

Methods

In order to determine if different observers have an effect on the assessment of invertebrate damage we conducted a cross calibration analysis. This involved the combined effort of five different observers, with different levels of expertise on the assessment of leaf damage by invertebrate herbivores: two expert observers, Sarah Rheubottom (SR) and Isabel Barrio (IB), and three undergraduate students with less experience in leaf damage assessment, Habba Mahal (HM), Michelle Goonasekera (MG), and Alexander MacKenzie (AM). Each of the three students used their results to complete individual undergraduate projects. All observers followed the same procedure for leaf damage assessment, in which leaves were categorized by damage type (external, mining, or gall) and by the percent area damaged (<1%, 1-5%, 5-25%, 25-50%, 50-75%, and >75%; **see Chapter 3**). In order to perform the cross calibration the same samples were assessed by multiple observers (**Table A2.1**). Samples contained ~100 leaves. Plant species used in this analysis included *Vaccinium uliginosum*, *Salix polaris, Dryas* *octopetala, Betula nana, Vaccinium myrtillus,* and *Arctous alpina*. Four observers examined the same four samples of *V. uliginosum*, three observers examined the same five samples of *S. polaris*. Two observers examined the same seven samples of *D. octopetala*. Two observers examined the same five samples of *Betula nana, Vaccinium myrtillus,* and *Arctous alpina*. Observers were unaware of leaf scorings by the other observers.

Table A2.1 Combinations of observers, plant species and number of samples used for the cross-calibration of observer effects on leaf damage assessments. Samples include ~100 leaves of an individual plant. SR: Sarah Rheubottom, MG: Michelle Goonasekera, IB: Isabel Barrio, HM: Habba Mahal, AM: Alexander MacKenzie.

Species	Observers				Number of samples
Vaccinium uliginosum	SR	MG	HM	AM	4
Salix polaris	SR	MG	HM		5
Dryas octopetala	SR	MG			7
Betula nana	SR	IB			5
Vaccinium myrtillus	SR	IB			5
Arctous alpina	SR	IB			5

Response variables included the proportion of leaves damaged (PLD) and the percent leaf area damaged (PLAD; **see Chapter 3 for calculations**). Linear mixed effects models (LMM) were used to determine if the observer had an effect on the assessment of PLD and PLAD for total, external, mine, and gall herbivory. Separate models were built for each of the six plant species and response variables. Observer was included as a fixed effect variable, and to account for the study design (i.e. several assessments for each sample), sample ID was included as a random effect. We used the Log-Likelihood Ratio test (LRT) to assess the overall significance of observer, comparing the models with and without observer as a predictor variable.

To assess if the effect of observer varies across plant species, we used a subset of the samples that included 3 species that had been scored by the same two observers (SR and MG). This LMM included the interaction between observer and plant species as a fixed effect, and sample ID was included as a random factor. As well, as a measure of repeatability, the intra-class correlation was calculated for each response variable (**Table A2.2**). The intra-class correlation allows assessing the degree of similarity between observers examining the same leaf samples. A high intra-class correlation coefficient indicates a high degree of similarity.

Table A2.2 Intra-class correlation values for the percent leaves damaged (PLD) and percent leaf area damaged (PLAD) for each of the species' included in the analysis. Total: all herbivore damage. External: external herbivore damage. Mine: mining damage. Gall: gall damage. Values less than 0.40 are marked with an *, and indicate a poor intra-class correlation. No mines or galls were recorded for *Betula nana* and *Vaccinium myrtillus*.

Species	Total	External	Mine	Gall
Vaccinium uliginosum				
PLD	0.63	0.66	0.49	0.35*
PLAD	0.92	0.92	0.61	0.24*
Salix polaris				
PLD	0.60	0.32*	0.48	0.00*
PLAD	0.37*	0.38*	0.48	0.00*
Dryas octopetala				
PLD	0.93	0.88	<0.001*	0.00*
PLAD	0.95	0.94	<0.001*	0.00*
Betula nana				
PLD	0.95	0.95	--	--
PLAD	0.96	0.96	--	--
Vaccinium myrtillus				
PLD	0.90	0.90	--	--
PLAD	0.94	0.94	--	--
Arctous alpina				
PLD	0.98	0.98	--	--
PLAD	0.95	0.95	--	--

Results

Vaccinium uliginosum

The assessments made by 4 different observers on *Vaccinium uliginosum* (**Figure A2.1**) did not significantly differ for PLD with any herbivore damage (LRT=3.06, p=0.38), with external damage (LRT=3.99, p=0.26), with mining damage (LRT=3.44, p=0.33), or gall damage (LRT=3.67, p=0.30). Similarly, the observer had no significant effect on PLAD by all herbivores (LRT=0.46, p=0.93), external damage (LRT=0.55, p=0.91), leaf miners (LRT=3.63, p=0.30) or gallers (LRT=3.75, p=0.29).

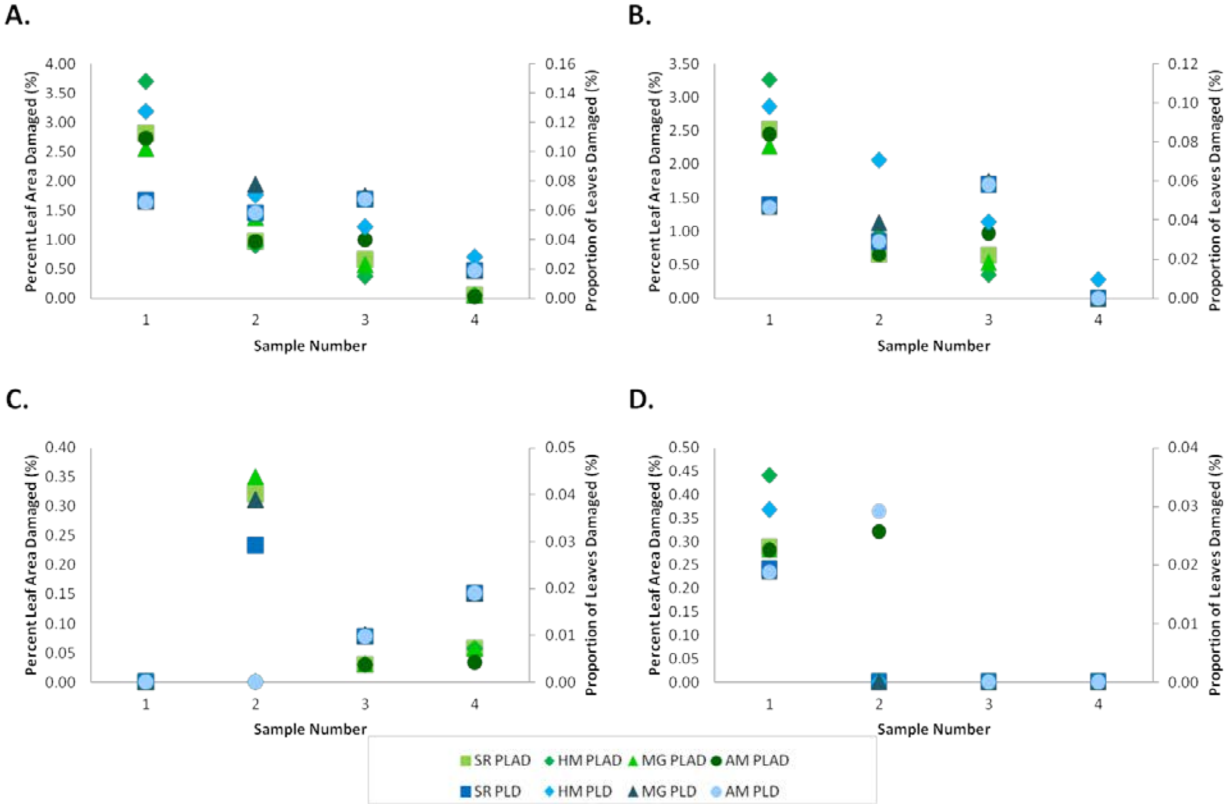


Figure A2.1 Measures of herbivory on *Vaccinium uliginosum* conducted by different observers. A: Total herbivory. B: External herbivory. C: Gall damage. D: Mine damage. Figures show the percent leaf area damaged (PLAD) with green icons, and the proportion of leaves damaged (PLD) with blue icons. The four observers scored the same four samples.

Salix polaris

Three observers examined the same five samples of *S. polaris* (**Figure A2.2**). Observer had a significant effect on the PLD with total herbivore damage (LRT=10.11, p=0.006), the PLD with external herbivore damage (LRT=6.41, p=0.04), and the PLD with gall damage (LRT=7.35, p=0.03). However, the observer had no significant effect on the PLD with mining damage (LRT=5.56, p=0.062) or on the PLAD by external damage (LRT=4.92, p=0.09), or by leaf miners (LRT=4.94, p=0.08). Lastly, the observer did have a significant effect on the PLAD by total herbivory (LRT=6.11, p=0.047), and by gallers (LRT=7.35, p=0.03).

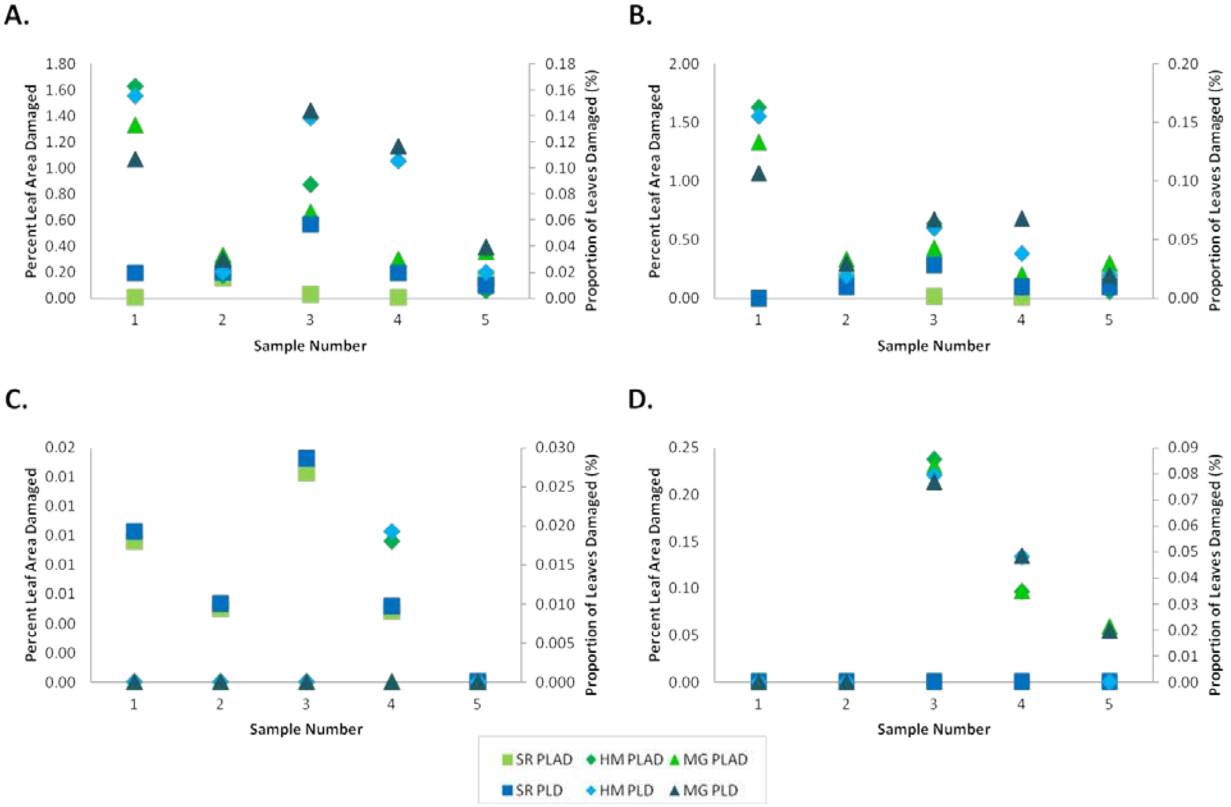


Figure A2.2 Measures of herbivory on *Salix polaris* conducted by different observers. A: Total herbivory. B: External herbivory. C: Gall damage. D: Mine damage. Figures show the percent leaf area damaged (PLAD) with green icons, and the proportion of leaves damaged (PLD) with blue icons. The three observers scored the same five samples.

Dryas octopetala

The assessments made by two observers on *Dryas octopetala* were not significantly different for majority of the herbivory measurements (**Figure A2.3**): the PLD with total herbivore damage (LRT=1.97, p=0.16), with external herbivore damage (LRT=1.85, p=0.17), with mining damage (LRT=2.23, p=0.13) or with gall damage (LRT=3.07, p=0.08). The observer did not have a significant effect on the PLAD specifically affected by external feeders (LRT=3.19, p=0.07), leaf miners (LRT=1.29, p=0.26) or gall damage (LRT=2.95, p=0.09), but the observer was found to have a significant effect on the total herbivore damage PLAD (LRT=4.46, p=0.03).

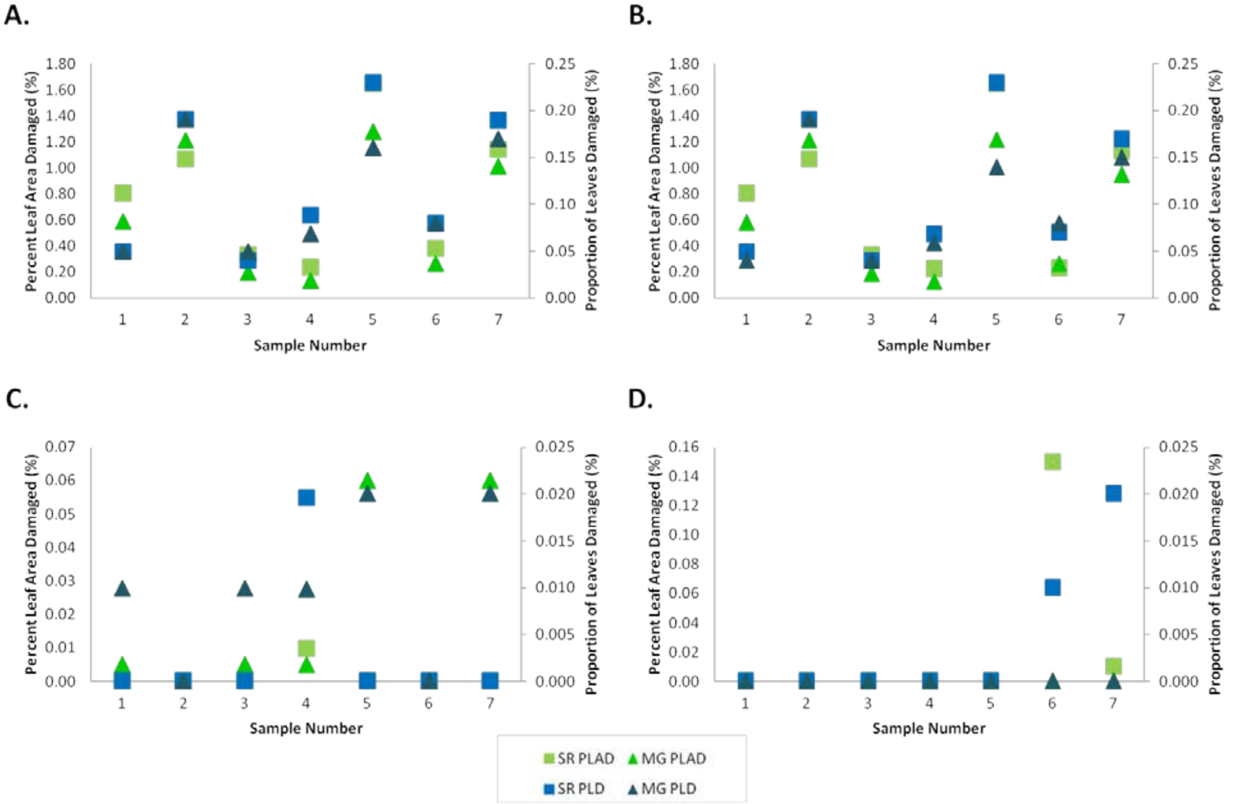


Figure A2.3 Measures of herbivory on *Dryas octopetala* conducted by different observers. A: Total herbivory. B: External herbivory. C: Gall damage. D: Mine damage. Figures show the percent leaf area damaged (PLAD) with green icons, and the proportion of leaves damaged (PLD) with blue icons. The two observers scored the same seven samples. Note that the main difference between A and B occurs on sample 6 (resulting in the significant observer effect on total PLAD).

Betula nana

There was no significant difference between the assessments of the two observers of *Betula* nana (**Figure A2.4**). PLD for total herbivory and external herbivory were not significantly different (LRT = 1.91, p= 0.17 and LRT= 1.91, p=0.17, respectively). PLAD for total herbivory and external herbivory were not significantly different either (total herbivory: LRT= 0.07, p=0.79; external herbivory: LRT= 0.07, p=0.79). Both observers recorded zeros for mine and gall damage so only the PLD and PLAD for total and external damage were examined.

Vaccinium myrtillus

Observer did not have a significant effect on the assessment of leaf damage on *Vaccinium myrtillus* (**Figure A2.4**). Neither PLD nor PLAD for total herbivory and external herbivory significantly differed between observers (PLD total herbivory: LRT= 0.17, p= 0.68; PLD external herbivory: LRT= 0.17, p= 0.68; PLAD total herbivory: LRT= 0.84, p=0.36; PLAD external herbivory: LRT= 0.84, p=0.36). Both observers recorded zeros for mine and gall damage so only the PLD and PLAD for total and external damage were examined.

Arctous alpina

The assessments made by two observers on *Arctous alpina* were not significantly different for any of the herbivory measurements (**Figure A2.4**): the PLD with total herbivore damage (LRT= 2.25, p=0.13), with external herbivore damage (LRT= 2.25, p=0.13). The observer did not have a significant effect on the PLAD with total herbivore damage (LRT= 0.41, p=0.52), with external herbivore damage (LRT= 0.41, p=0.52). Since both observers reported zeros for mining and gall damage no analysis was conducted for these feeding types.

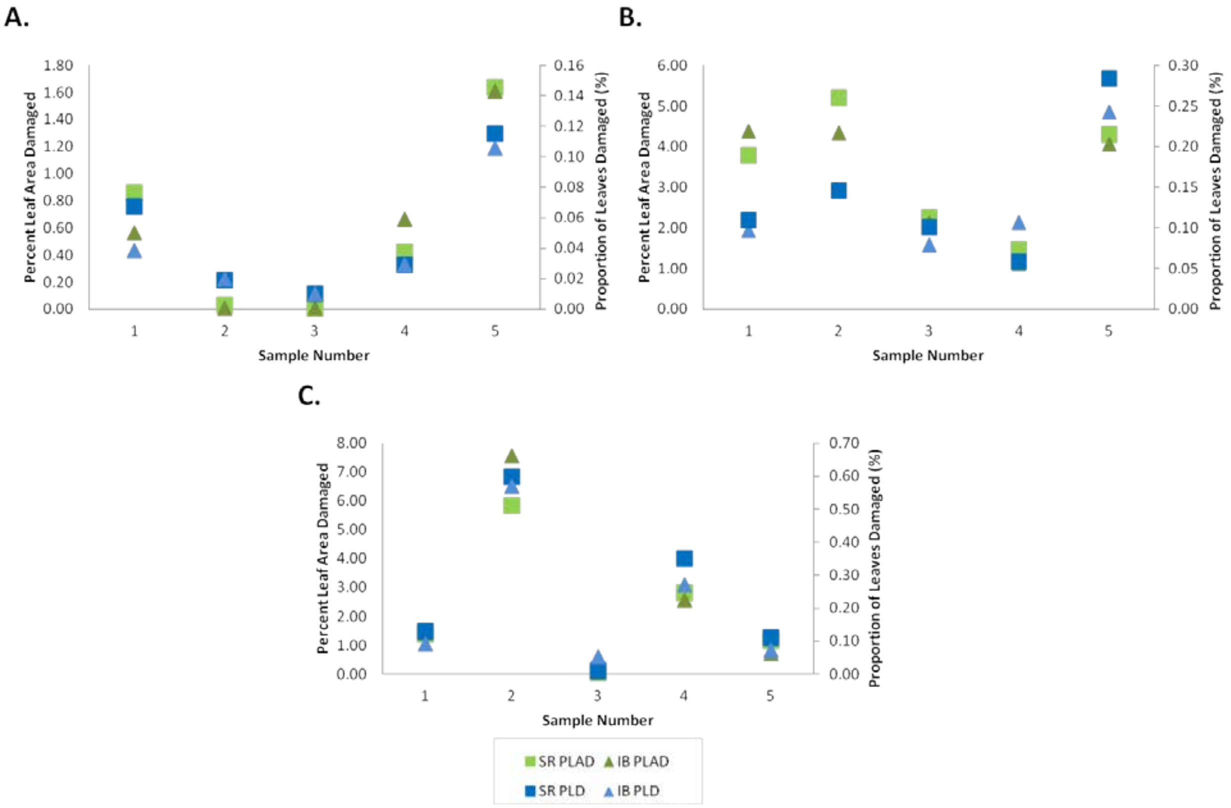


Figure A2.4 Measures of herbivory on 3 species conducted by two different experienced observers. A: *Betula nana* B: *Vaccinium myrtillus* and C: *Arctous alpina*. Both observers only reported external damage for all three species, therefore only total herbivore damage is shown. Figures show the percent leaf area damaged (PLAD) with green icons, and the proportion of leaves damaged (PLD) with blue icons. The two observers scored the same five samples for each species.

Species-specific effect

When comparing the assessments of two observers of samples of three of the species, we found that the effect of observer depended on the plant species being analysed (observer*species; LRT=16.27, p<0.001; **Figure A2.5**). This effect was driven by the significant differences in the assessments of the two observers for *Salix polaris*, but no differences in the assessments for the other two species.

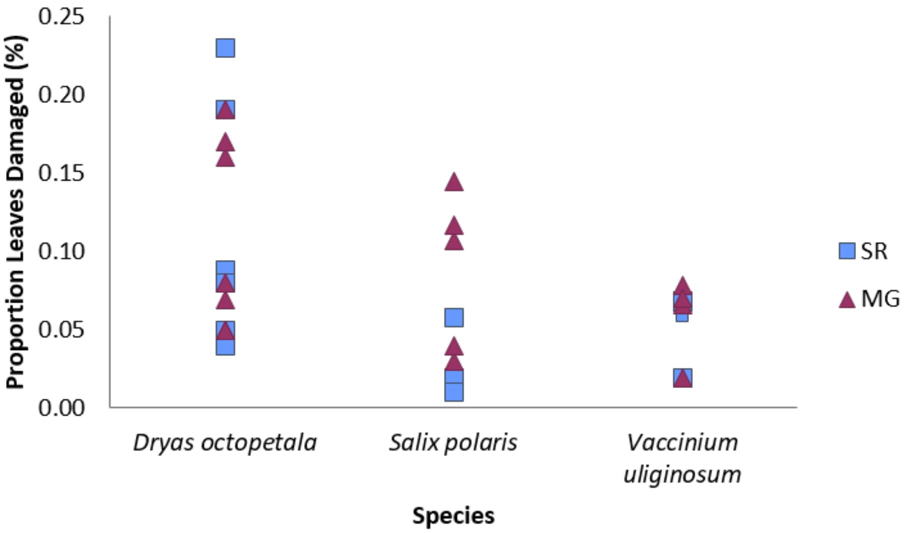


Figure A2.5 Assessment of leaf damage by two observers (SR, MG) on three plant species: *Vaccinium uliginosum* (4 samples), *Salix polaris* (5 samples), and *Dryas octopetala* (7 samples). Each icon represents one of the samples for that species, with the colour and shape corresponding to the observer. High overlap of the two different icons indicates high similarity in observer assessment. The two observers scored the same samples.

Discussion

The results of the cross calibration indicate that there is an effect of different observers on the assessment of invertebrate herbivory for some of the species analysed (*Salix polaris* and *Dryas octopetala)* but not others (*Vaccinium uliginosum, Vaccinium myrtillus, Arctous alpina,* and *Betula nana*).

The differences with *S. polaris* were detected with the assessment of the total number of leaves that showed some form of invertebrate herbivory, the number of leaves with external damage and gall damage, and the percent area damaged by total herbivory and by galls. *S. polaris* is a small shrub with small round leaves (5-32 mm long and 8-18 mm wide; Aiken et al. 2007). In the process of drying and preservation of leaves many of these leaves cracked along the midrib at the tip of the leaf (**Figure A2.6**). This mechanical damage can easily look like chewing damage and that may have led to a skewed analysis by some observers.

Figure A2.6 Image of *Salix polaris* growing in the wild (Arkkio 2004) next to a dried leaf. The split in the dried leaf occurs due to the flattening of the round, slightly curled leaves of this species. This split may be erroneously classified as invertebrate herbivory.

With regards to *D. octopetala,* observer had a significant effect on the PLAD by total herbivory. Similar to the *S. polaris* leaves, this species was susceptible to overestimation based on the lobed morphology of the leaf, resulting in lobes being broken off by mechanical means and being classified as chewing damage.

These results are similar to those found by Johnson et al. (2016), where they found that plant species can have an effect on how damage is assessed. Johnson et al. (2016) also found that observer experience also affected the level of herbivory estimated; with inexperienced observers overestimating damage levels. This is consistent with our results as well since within *Salix polaris* both of the inexperienced observers overestimated the extent of damage compared to the expert observer. This could mean that the effect of observer could be corrected by including more training for complicated species.

All of the leaf damage assessments in the present study were conducted by the same observer (SR). Based on the experience of assessing the 45 focal plant species, there are 6 species (other than *S. polaris* and *D. octopetala*) that may have a more complicated damage assessment. This includes *Salix arctica, Papaver radicatum, Carex aquatilis, Carex bigelowii,* and *Carex sp.* mainly because the damage occurring on these leaves is distributed in spots all around the leaf making it harder to assess the overall percent area damaged. For these graminoid species another added issue is that the whole leaf cannot be seen in one view, again this makes determining the overall percent area damaged difficult. Lastly, *Oxyria digyna* may also present some problems if the leaves are not preserved properly since they can form bubbles which can be misidentified as mining damage. The analysis only included leaves from shrubby species, and the inclusion of graminoid species may have made the observer effects more pronounced due to the reasons outlined above. These results lead me to conclude that for the most part leaf analysis can be completed by multiple observers, with the stipulation that complex leaves are either all assessed by the same observer, or have a guide to common errors in assessment.

Appendix 3: Point-intercept to Biomass Conversion

The conversion of the point-intercept hits to biomass was based on the methods outlined by Bråthen and Hagberg (2004). Point intercept data was collected for all 16 frames for each focal species, within those 16 frames 3 had all above ground biomass collected as well (**Figure 3.1**). Point intercept frames utilized 10 pins and were 50 cm x 50 cm. The biomass collected from the 3 frames in combination with the point hits of those frames were used to determine a conversion factor (CF; Eq. 1). These conversion factors were then used to convert the point hits into a biomass estimate for the frames that did not have biomass collected (Eq. 2).

$$CF = \frac{\sum Focal\ species\ biomass}{\sum\left(\frac{Number\ of\ hits}{Number\ of\ points\ in\ the\ frame}\right)} \qquad \text{Eq 1.}$$

$$Biomass = CF\left(\frac{Number\ of\ hits}{Number\ of\ points\ in\ the\ frame}\right) \qquad \text{Eq. 2}$$

Conversion factors were calculated for each focal species with the exception of a few that needed to be combined into groups due to insufficient biomass samples: G1 *Salix arctica* and *S. reticulata*; G2 *Silene acaulis, Armeria maritima,* and *Saxifraga oppositifolia*; G3 *Luzula confusa* and *L. spicata*; G4 *Juncus trifidus* and *Trichophorum cespitosum*; G5 *Molinia caerulea* and *Helictochloa versicolor*; G6 *Saxifraga hyperborea, Cochlearia groenlandica,* and *Oxyria digyna*; G7 *Petasites frigidus, Bistorta vivipara*, *Cerastium alpinum*, and *C. arcticum*; G8 *Poa arctica* and *Festuca rubra*; G9 *Betula nana* and *Rhododendron tomentosum.*

Table A3.1 The conversion factors for the focal species examined. The conversion factor is used to convert point-intercept data to biomass estimates.

Species	Conversion Factor	Species	Conversion Factor
Andromeda polifolia	10.04	*Luzula spicata*	11.94
Arctagrostis latifolia	9.73	*Molinia caerulea*	5.07
Armeria maritima	44.74	*Oxyria digyna*	7.63
Avenella flexuosa	5.53	*Petasites frigidus*	42.61
Betula nana	33.13	*Poa arctica*	8.17
Bistorta vivipara	42.61	*Primula integrifolia*	8.27
Calluna vulgaris	32.59	*Rhododendron tomentosum*	33.13
Carex aquatilis	5.27	*Salix arctica*	22.92
Carex bigelowii	14.27	*Salix herbacea*	11.82
Carex sp.	9.02	*Salix polaris*	12.15
Cerastium alpinum	42.61	*Salix pulchra*	43.33
Cerastium arcticum	42.61	*Salix reticulata*	22.92
Cochlearia groenlandica	7.63	*Silene acaulis*	44.74
Dryas octopetala	29.24	*Saxifraga hyperborea*	7.63
Empetrum nigrum	47.68	*Saxifraga oppositifolia*	44.74
Eriophorum vaginatum	3.42	*Salix rotundifolia*	3.07
Festuca rubra	8.17	*Trichophorum cespitosum*	3.42
Helictochloa versicolor	5.07	*Vaccinium myrtillus*	23.43
Juncus trifidus	3.42	*Vaccinium uliginosum*	17.45
Kalmia procumbens	28.49	*Vaccinium vitis-idaea*	18.95
Luzula confusa	11.94		