Characteristics of alpine plants and soils along an elevational gradient, Northern Selkirk Mountains, British Columbia

by

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Abstract

Rates of climate change are accelerated at higher elevations, a pattern termed elevation-dependent warming (EDW). Consequently, the impacts of climate change on community patterning and soil development may be particularly evident in alpine environments. Alpine ecotone boundaries, such as treeline and glacier margins, are generally thought to be shifting to higher elevations. Soil properties, both physico-chemical and microbial, and soil development will likely determine the rate of both treeline advance and alpine plant movement into recently-deglaciated terrain. Soil gaseous fluxes will also influence the extent of future climate change globally and are not well understood in alpine ecosystems.

I documented the current state of alpine plant community composition and soil properties along an elevational gradient, the effects of soil properties on plant species establishment in recently-deglaciated terrain, and the direction, magnitude, and mechanistic drivers of methane and carbon dioxide soil gaseous fluxes in an alpine valley in the Northern Selkirk Mountains, British Columbia.

Local scale plant community composition was more strongly related to local soil properties than elevation. Soil development, associated with ongoing climate change, will likely have a large effect on community composition. I found no evidence of treeline advance occurring within at least the last decade based on the age structure of sub-alpine fir across the treeline ecotone.

Three out of four transplanted later-successional alpine species were able to survive and grow in recently-deglaciated terrain. Survival was either due to recently-deglaciated terrain acting as a selection factor for a subset of individuals or chance placements in appropriate

microsites for graminoid species. *Artemisia norvegica* had a relatively high survival but reduced growth compared to control individuals indicating that establishment of this species may be a slow process.

CH_4 uptake was observed across all sites and was most significant for alpine and mid elevation sites. CH_4 uptake was primarily driven by soil moisture (as a reverse proxy for aeration), total carbon, and soil texture. Significant methane fluxes were only found in sites with developed soils, relatively high plant cover, and good drainage which are all expected to increase with climate change forming a potential negative feedback loop. Soil CO_2 emissions were highest in sites with high plant cover, high NO_3^- levels, and enriched $\delta^{15}N$. However, these sites were likely to have large uptake of CO_2 via photosynthesis by vegetation potentially offsetting or reversing the CO_2 emissions as suggested by the accrual of soil organic carbon in the sites with high plant coverage.

Current soil properties may be limiting treeline advance and have a strong relationship with alpine vegetation communities. The relatively slow development of soil properties may also limit the movement and establishment of herbaceous alpine vegetation into recently-deglaciated terrain, however, there is a potential for some species to survive in these areas. Soil methane uptake in the alpine environment helps to offset the general trend of greenhouse gas emission globally, although uptake is lower than emissions in many other ecosystem types (e.g., boreal wetlands). Climate change effects seem to reinforce methane uptake in alpine soils and will lead to a negative feedback in these areas.

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Chapter 1

Introduction

Climate change is occurring at an accelerated rate in both high elevation and high latitude environments, at a rate nearly double that of the global average (Rangwala and Miller 2012, Spasojevic et al. 2013, Pepin et al. 2015). Climate change affects more than just the temperature. There are also direct and indirect influences on precipitation, vapour pressure, solar radiation, and other climatic variables, which in turn influence large scale landscape patterns and processes, including plant communities, succession, soil properties, and soil development. Many changes are already being observed in alpine areas, including glacial recession, reduced snowpack, and altered water flow dynamics (e.g. Klanderud and Birks 2003, Barry 2006, Ward 2011, Spasojevic et al. 2013). The effects these changes have on the landscape is varied and still not well understood. For example, the established ranges of plant species are likely to shift as a result of climate change (Klanderud and Birks 2003, Schöb et al. 2009). However, plant responses to warming are somewhat unpredictable, and depend on the scale of study and type of warming (Alatalo et al. 2016). For this reason, plant responses need to be measured at a variety of scales (local to regional) along with a plethora of climate factors to determine the complex interactions between the variables (Yang et al. 2018).

Ecotone boundaries in alpine areas, such as treeline and glacial extent, are more easily observable indicators of climate change effects on the landscape and their movement over time can be quantified at coarse scales. Glacial recession exposes new terrain that can be colonized by early successional plant species and the length of the deglaciation period corresponds to the local plant community. In mid-altitude mountains in northeastern North America, after late-glacial and

Holocene colonization of tundra plants it took nearly 1,000 years for the vegetation to shift to partially forested (Martin and Germain 2016). Since anthropogenic climate change is inducing species to shift their ranges it is possible that the rate of succession will increase in alpine ecosystems causing a quicker transition to forested systems. Treeline has been observed to be moving upslope in some alpine regions, known as treeline advance, which may displace established alpine vegetation communities already present (Körner 2003, Holtmeier and Broll 2007, Richardson and Friedland 2009). Other studies show potential treeline infilling where there are more trees growing in areas already amenable to growth below treeline increasing tree density (Camarero et al. 2017).

Similarly, it is possible that traditionally later-successional alpine tundra species will have more access to recently-deglaciated terrain as it becomes more exposed due to glacial recession and shift their natural ranges to compensate for the environmental changes brought on by climate change. If these later-successional species can become established in recently-deglaciated areas, it may alter community assembly through succession as the initial colonizing species determines the subsequent species that establish via biotic processes such as competition and facilitation; essentially the colonizer determines the seral stages that proceed after it (Zhang et al. 2015). If the initial colonizers are now later-successional species, the entire successional pathway may change as these species will have different reciprocal interactions with the soil (plant-soil feedbacks) which can potentially alter the physico-chemical properties of the soil as it develops.

Soil is another factor that sets apart early-successional and later-successional areas on a mountain. Following the Canadian System of Soil Classification (Soil Clasification Working Group 1998) recently-deglaciated soils are generally classified as Regosolic and likely develop into Brunisols over time. The soil properties associated with recently-deglaciated Regosolic soils are different from those in more developed soils. Soil formation and development is controlled by five primary factors: time, topography, organisms, climate, and parent material. Recently-deglaciated soils have not had a long period to develop and contain little biotic material. Comparatively, Brunisols have had longer development times and generally contain more plant biomass and different proportions of microbial groups, such as bacteria and fungi. These key differences between the most common soils types in these high alpine ecosystems can act as a barrier to plant establishment in recently-deglaciated soil as many species are not adapted to the lack of nutrients and microbial community as generally found in Regosols (Müller et al. 2016, Zeng et al. 2016). The ultimate differences in soil forming factors between Regosols and Brunisols leads to differences in proximate physico-chemical properties of the soil. Dubuis et al. (2013) found that pH and nitrogen differences in soils along an elevational gradient are likely drivers of plant establishment in the Alps. The change in chemical properties between recently-deglaciated soils and more developed soils will determine which plant species can establish, grow, and survive in the recently-deglaciated terrain. Soil properties are expected to change with ongoing climate change, as well as increased atmospheric nitrogen deposition (Shaw and Harte 2001, Fenn et al. 2003), which may simultaneously alter which plant species are able to thrive in different areas over time.

Soil nitrogen has high spatial variability locally, and the amount of inorganic nitrogen within soil varies along a successional gradient (Gross et al. 1995). Current levels of nitrogen deposition in North American temperate mountains is estimated to be approximately 7 kg N ha^{-1} yr^{-1} (Burns 2003), and this value is likely to increase due to increased anthropogenic nitrogen fixation (Galloway et al. 1994, Baron et al. 2000, Zavaleta et al. 2003). Anthropogenic sources of nitrogen fixation currently exceed natural nitrogen fixation and will likely increase as the global population continues to grow (Galloway et al. 1994, Zavaleta et al. 2003). Under simulated warming conditions the rates of nitrogen mineralization nearly doubled dependent on the moisture of the site, with xeric sites having the largest increase; immobilization also increased temporarily (Shaw and Harte 2001). Soil moisture is generally not limiting in alpine environments except in low precipitation years (Bowman et al. 1995). Generally, soil moisture availability is not a precluding factor for vegetation establishment and growth in the alpine, however, it still affects community assembly at different locations on a mountain. The interaction between soil moisture and nitrogen mineralization may therefore mediate climate-induced increases in nitrogen mineralization and immobilization rates. However, the complex interaction between climate change and soil moisture directly has the potential to both exacerbate or limit nitrogen mineralization increases. Warming conditions will likely decrease soil moisture in alpine areas (Harte et al. 1995), but climate change increases the likelihood of extreme precipitation events which might allow soil moisture to stay non-limiting in these areas (Meißl et al. 2016), depending on factors such as the water storage capacity of the soil profile.

A larger nitrogen pool due to climate change and atmospheric deposition may alleviate nitrogen limitation in recently-deglaciated soils for later-successional plant species. However, the ability of recently-deglaciated soils to increase their inorganic nitrogen pools is influenced by their ability to increase their nitrogen-fixing microbial population. Microbes associated with nitrogen cycling generally increase in abundance along a successional gradient (Zeng et al. 2016). Nitrogen is also tied to pH dynamics of soil over time, where increased nitrogen deposition leads to lowering of soil pH (Bowman et al. 2008). Soil pH decreases naturally over time, generally attributed to increases in plant biomass, which often release acidic root exudates.

This highlights the potential of an additive effect or multiplicative interactions between soil properties over time, which may be exacerbated by climate change.

Soil microbial community composition has a reciprocal interaction with plant species, where certain microbe functional groups may enhance the establishment and abundance of plant species and the presence of plants facilitate the abundance of certain microbes (Chapin et al. 1994, Tscherko et al. 2005). Studies have found that soil microbes decrease in abundance and diversity as elevation increases (Djukic et al. 2010, Xu et al. 2014), whereas others show no trend in diversity across elevation (Shen et al. 2014). Contrastingly plant diversity is generally found to be highest at intermediate elevations (Bryant et al. 2008), showing a disconnect in the properties that drive microbial and plant diversity. Interestingly, Archaea generally have the highest abundance and diversity at high elevation early successional sites (Esperschütz et al. 2011). The relative abundance of different microbial groups change with elevation (Margesin et al. 2009, Djukic et al. 2010, Esperschütz et al. 2011, Xu et al. 2014), these changes can have large effects on the plant species that thrive at a given elevation. Fungi, particularly mycorrhizal fungi, are largely beneficial to plants, and many species rely on mycorrhizal fungi to grow. Bacteria to fungi ratio typically decreases at lower terrain elevations indicating relatively more fungi at those locations (Djukic et al. 2010, Xu et al. 2014). If plants from those lower, more developed mountain areas rely on fungi to thrive, they may be less able to establish and move into alpine and recently-deglaciated areas dominated by Archaea and bacteria. Microbial community is also influenced by soil physico-chemical properties, especially pH and C:N, which change with elevation (Högberg et al. 2007, Bardelli et al. 2017). Climate change may also alter the microbial community dynamics, primarily believed to decrease microbial abundance by altering the conditions the community is best adapted to (Gavazov et al. 2017, Puissant et al.

2017). The interactions between the microbial community and the environment will likely have an effect on the ability of plant species to alter their elevational distributions.

Although climate change will affect both soil physico-chemical and microbial properties as well as vegetation communities in the alpine, these variables may also impact soil greenhouse gas fluxes which could potentially negatively feedback on climate change in the area lessening its effects (Lin et al. 2017). CH_4 and CO_2 are two common greenhouse gases contributing to climate change that are found in soils which contribute to a significant amount of greenhouse gases globally (Dutaur and Verchot 2007, Oertel et al. 2016, Lin et al. 2017). Gas fluxes are driven by both microbial and physical soil properties as microbes are responsible for the production or breakdown of these gaseous compounds while pore size distribution and moisture are responsible for the ability of these gases to diffuse across the soil-air interface as they determine the relative concentrations of these gases across the gradient (Smith et al. 2003, Hofmann et al. 2016b, 2016a, Oertel et al. 2016). Changes in microbial community and soil physico-chemical properties in an area will alter the strength and direction of the soil fluxes. Methane emission and uptake is controlled by the amount and activity of methanogenic and methanotrophic bacteria in the soil, where more of one type will determine whether there is a net uptake or release of methane (Hofmann et al. 2016b). The balance of these bacteria types is not well known in recently-deglaciated terrain in North America. Carbon dioxide emission and uptake is controlled by the balance between cellular respiration from the microbial community and plant roots and the uptake of CO_2 by plants from the atmosphere (Raich and Schlesinger 1992, Berg and Smalla 2009, Lin et al. 2017). CO_2 fluxes from between the soil-air interface specifically will mainly be determined by the amount of vegetation in an area (which will also determine the microbial population and activity through the rhizosphere), soil temperature, and the amount of carbon available for the microbial community (Saleska et al. 1999, Gulledge and Schimel 2000, Kitzler et al. 2006). pH, C:N ratio, and soil moisture have also been found to be strong drivers of soil fluxes, and of methane in particular (Saleska et al. 1999, Koch et al. 2007, Dubuis et al. 2013, Hofmann et al. 2016a). Understanding the strength of gas fluxes across the alpine zone and the drivers of these fluxes is essential for understanding how greenhouse gas production will feedback into climate change in these regions (Dutaur and Verchot 2007). This can also provide an understanding of how the fluxes will change into the future based on the current climate change effects already known.

The reciprocal interactions between climatic and edaphic conditions on alpine plant distribution in the face of climate change can be explored by investigating how plant communities and soil factors change together along an elevational gradient, as well as determining the ability of later-successional plant species to grow and survive in recently-deglaciated terrain and observing large scale ecotone boundary shifts. Plant communities vary along altitudinal gradients due to the different optimal elevation range for each species, and biotic and edaphic interactions between species (Bruun et al. 2006). Communities change in species composition as one goes up in altitude on a large spatial scale, however local scales are likely more chaotic with less defined patterns. Biotic interactions between species in a community include both competition and facilitation (Mcintire and Fajardo 2014), and the strength of these interactions has been found to change with increasing stress (Grassein et al. 2014) along altitudinal gradients in this case.

Species' optimal elevations are influenced by biotic, climate, and soil conditions associated with different altitudinal bands (Brown and Vellend 2014), therefore, elevational changes in soil factors will affect plant community composition. Determining the correlation between various soil parameters and community composition along an elevational gradient will provide insight into which soil parameters have the largest influence on plant communities in alpine ecosystems. Exploring the spatial pattern of soil physico-chemical properties along elevation can provide insight into spatial distribution and concurrence of plant species in alpine areas. The baseline data of how plant community composition changes along elevation and why will allow for inference about how individual species distribution and community composition may change with climate change in North American temperate alpine areas. Shifts in treeline are observable using dendrochronological methods to construct chronosequences of alpine tree age distributions (Szeicz and Macdonald 1995). Ratios between old and young trees can provide insight into the potential for treeline to be advancing upslope into higher elevations, or if it is infilling or stagnant at all elevational floors. Treeline advance can have drastic consequences on alpine communities as trees will displace alpine species and alter the soils and microsites around them, often making them unsuitable for the previous species. Determining if treeline has recently advanced up in mountain slopes is important for anticipating the future of the alpine communities found in these environments.

One potential barrier to the upward movement of plant ranges is the suitability of the soil compounded with harsh weather conditions in high elevation sites (Matthews 1992, Dubuis et al. 2013). With climate amelioration driving upward distributional shifts, later-successional plant species will have greater potential to establish and grow in sites that were previously outside of their tolerance limits, at least regarding climatic conditions. Whether these later-successional species can survive in relatively inhospitable recently-deglaciated terrain is not well understood. Transplant experiments are an effective way to investigate the effects of soil conditions and other potential drivers of distribution on alpine plant species (Grassein et al. 2014, Alexander et al.

2015). Transplanting later-successional species to recently-deglaciated sites allows investigation of natural variation in soil conditions between recently and later deglaciated sites and their ability to slow or prevent changes in species distribution. High elevations correspond to lower nitrogen deposition (Fenn et al. 2003) and greater area of recently deglaciated soils, therefore, it is likely that these areas will have less available nitrogen compared to later deglaciated sites. A nitrogen addition treatment can be an effective way of investigating the effects of removing this potential nutrient limitation in alpine areas (Gough et al. 2000). If later-successional species are benefitted by the additional nitrogen supplemented to the recently-deglaciated soil, they will respond with higher growth or survival in these areas.

Soil methane and carbon dioxide fluxes from alpine regions are not well known, in particular in Canada. The majority of studies on soil fluxes in temperate mountain regions occurred in Europe, United States, and China (Brooks et al. 1997, Kitzler et al. 2006, Koch et al. 2007, Zhu et al. 2015, Knowles et al. 2015, Wei et al. 2015, Hofmann et al. 2016a, Chiri et al. 2017, Wu et al. 2017, Lin et al. 2017, Fu et al. 2018, Mutschlechner et al. 2018). Greenhouse gas fluxes are vitally important to quantify and understand for climate change as they are the mechanism that drives global warming (Smith et al. 2003, Dutaur and Verchot 2007, Tian et al. 2016). Soil greenhouse gas exchange with the atmosphere can either exacerbate or diminish climate change effects by feedback interactions with the vegetation and soil attributes. Warming temperatures and changing moisture regimes can shift the balance of emission and uptake for both methane and CO_2 as they alter the vegetation and soil communities that are responsible for these fluxes (Torn and Harte 1996, Koch et al. 2007, Wei et al. 2015, Oertel et al. 2016, Lin et al. 2017). Determining the mechanisms that drive soil fluxes in the alpine will help to identify what parameters are most important for determining the feedback effects on climate change and how they will likely change with current climate projections. The variation in vegetation communities and soil physico-chemical properties will determine the balance of CH_4 and CO_2 fluxes in recently-deglaciated terrain, alpine grassland, and along an elevational gradient towards treeline. The soil fluxes and their mechanistic drivers found in these areas will add to global climate models to better understand the effects climate change will continue to have in the future, both in the alpine and around the world.

Our study aims at integrating the three aspects of alpine community dynamics to gain a better understanding of the proportion, potential, and extent of alpine plant range shifts and the effects soil properties have on influencing plant species movement. The study site was located in Bachelor Pass in the Northern Selkirk range of the Columbia Mountains, British Columbia, Canada. The study site spans from montane forest to alpine heathlands and meadows in the pass, and then extends into glacial foreland with little vegetation coverage and Regosolic soils. The area is suitable for addressing the questions of how do soil properties affect alpine plant distribution, are later-successional alpine species able to grow and survive in recently-deglaciated areas, and what are the strength and mechanistic drivers of CH_4 and CO_2 soil fluxes in different vegetation and soil communities in temperate alpine environments?

Chapter 2

Relationships between soil properties and alpine plant community composition along an elevational gradient, North Selkirk Mountains, British Columbia

Introduction

The effects of climate change alter both biotic and abiotic conditions in mountain environments (Henry and Molau 1997, Bornman et al. 2015, Alatalo et al. 2016, Luo et al. 2017). Warming temperatures affect species optimal ranges which are expected to cause species to shift their ranges upslope (Chen et al. 2011). However, edaphic and topographic conditions are also important for determining whether a species can survive impacting community composition and biodiversity (Boyce et al. 2005, Bruun et al. 2006, Li et al. 2011, Takahashi and Murayama 2014, Yanyan et al. 2017). Climate change also affects soil aspects, such as CH_4 and CO_2 fluxes, atmospheric nitrogen deposition, and pH in an area, which will then further feedback into altering the biophysical conditions of the ecosystem.

The outcome of climate change on community composition will be determined by how the main drivers of community patterning are affected at different spatial and temporal scales (Alatalo et al. 2016). Alpine communities are generally warming faster than in adjacent lowland regions, a pattern termed elevation-dependent warming or EDW (Rangwala and Miller 2012, Spasojevic et al. 2013, Pepin et al. 2015). The ecological effects of EDW are still poorly documented and the impact of climate change on alpine communities require further study.

Ecotone boundaries are defined by sharp changes in vegetation communities over a short distance. Alpine treelines are one example of an obvious ecotone between montane forest and alpine tundra communities. Recent reports have shown that there is potential for treelines to advance upslope due to climate change ameliorating the previously harsher weather conditions above current treelines (Körner 2003, Holtmeier and Broll 2007, Richardson and Friedland 2009, Martin and Germain 2016). However, this advance is critically dependent on the suitability of soil conditions for supporting vegetation establishment above the current treeline (Davis and Gedalof 2018).

Subalpine habitats generally occur between montane forest and alpine and often encompass the treeline ecotone. Plant community patterning should change across this region from forested to alpine tundra/grassland communities along an elevational gradient. However, the cause of the change in community composition may be due to biotic and edaphic conditions rather than elevation and associated climatic factors (Kupfer and Cairns 1996). Since the ecotone between montane and alpine is relatively small, large-scale factors like elevation and climate may not drive the change at a local scale. It is important to characterize the range of variance in plant communities and edaphic conditions across ecotone boundaries to better understand how these areas may be potentially affected by ongoing anthropogenic climate change.

Topographic and edaphic property gradients in alpine regions have the potential to enhance or mitigate plant community composition shifts due to climate change (Suding et al. 2015). The topography of a landscape is a selection factor for what kinds of species can survive in an area, and has direct influence on plant communities as a result (Bruun et al. 2006). Topography also acts on plant communities through regulation of microclimate and through edaphic properties (Takahashi and Murayama 2014, Suding et al. 2015), which are more proximate mechanisms of plant community assembly. Soil properties, both physico-chemical and microbial, have been previously found to affect plant community composition (Dubuis et al. 2013, Van der Putten et al. 2013). These drivers of plant community patterning are also likely to change in magnitude and distribution with climate change; although, at longer time-frames than the plant communities themselves. Understanding their current distributions and function in community composition dynamics is essential to determine how climate change will affect plant communities indirectly through the soil.

Relationships between community composition and abiotic properties determine the magnitude of shift in community composition as a response to climate change. The strength of the relationships between various physico-chemical soil properties and topographic variables and the communities along an environmental gradient need to be well understood in order to determine how plant communities and soil properties will change in the future. This is particularly important across the ecotone boundary where the communities change over short distances. As treeline advances and glaciers retreat the fate of the alpine regions is less well understood. The rate of change on the landscape will determine how quickly the alpine communities will need to respond to these changes.

The objective of this study was to determine how plant community composition and soil properties co-vary across treeline along an elevational gradient. I aim at evaluating the range of variance in edaphic factors and community patterns along this gradient and determine how terrain elevation and soil properties are related to community composition across the treeline ecotone, encompassing montane, subalpine and alpine areas. I hypothesize that the vegetation communities have a stronger relationship with edaphic properties rather than elevation across the ecotone. I predicted that the communities will be more influenced by soil properties and that the properties most closely tied to plant community composition will change along the elevational gradient. A sub-goal of this study is to determine the distribution and age structure of a focal tree species along the elevation gradient to explore the potential for growth at different elevational bands in the montane and sub-alpine.

Methods

Study site

The study was conducted in Bachelor Pass (51° 31'21.06"N; 117° 57'31.55" W), located in the Northern Selkirks, one of four ranges that make up the Columbia Mountains, British Columbia. The field data for this study were gathered during the 2016 and 2017 growing seasons. The majority of the site characteristic data was gathered during the growth season of 2016, with additional supplementary data from the summer of 2017. Bachelor Pass was the location for the 2016 General Mountaineering Camp for the Alpine Club of Canada and is located approximately 72 km northwest of Golden, BC. The average elevation of the study site is approximately 2000 m a.s.l, spanning a range of 1900-2100 m a.s.l. The average yearly precipitation of the site from 1981-2010 was 319.02 mm according to PCIC High Resolution PRISM Climatology data (Pacific Climate Impacts Consortium, University of Victoria, and PRISM Climate Group, Oregon State University, (Jan. 2014). High Resolution Climatology. Downloaded from https://data.pacificclimate.org/portal/bc_prism/map/ on July 5, 2018). The average maximum and minimum temperatures from 1981-2010 were 3.69°C and -3.17°C, respectively for the pixels encompassing the site. Growing season (June-August) temperatures ranged from 4.27-12.51°C on average over the 1981-2010 period, with a mean precipitation of 143 mm over these three months. Herbivory levels are generally low within temperate alpine ecosystems (Diemer 1996, Scheidel and Bruelheide 2001). The study area is located in the Northern Columbia Mountains ecoregion within the Southern Interior Mountains ecoprovince

(Demarchi 2011). The vegetation of Bachelor Pass consists of two biogeoclimatic zones identified in the ecoregion: the Engelmann Spruce-Sub Alpine Fir Zone in the mid elevations, and the Interior Mountain-Heather Alpine Zone above approximately 2000 m elevation.

Site characterization

Transect lines were set up along the east facing slope of Bachelor Pass ranging from approximately 1900 m to 2100 m elevation. The range in elevation means the plots spanned from montane forest zone to alpine zone. The two transects were 100-130 m linear distance apart on the terrain slope. Plots were established every ten meters in elevation determined by global positioning system (GPS); the first transect contained a total of 20 plots and the second transect contained 19 plots. The slope of each plot was determined by placing a clinometer on the ground to measure the angle of the slope. The plant species contained in each plot were recorded along with the species abundance, mosses and lichens present were also recorded and grouped; referred to as "non-vascular species" in all subsequent analyses (Table 2.3). In each plot a 50x50 cm point frame quadrat was used to visually determine the percent cover of plant species present. The vegetation composition was used, along with observation of the overall landscape, to determine the habitat zone that each plot resides in (either: montane, subalpine, or alpine). The montane zone upper boundary is the treeline (highest elevation where tree growth forms are upright and exceed 2 m in height) (Wardle 1965, Körner 2003, Richardson and Friedland 2009). The subalpine zone upper boundary is the tree limit, defined as the elevation where the vegetation is solely comprised of prostrate, mat-forming, shrubs and herbaceous vegetation commonly associated with alpine tundra areas (Körner 2003). The alpine tundra habitat zone upper boundary is the snow line defined as the elevation where snow is persistent year round (referred to as the nival zone; Grabherr et al. 1995), which generally corresponds in our site to recently-deglaciated soils just below the glacier terminus, however the transects did not span across the mountain pass and therefore, did not enter the recently-deglaciated terrain. A third transect was established from the glacial terminus along a north facing slope into a valley. This third transect was approximately 900 m long with plots every 100 m linear distance. Each of the measurements collected at the original two transects were repeated for the third transect, to allow for the characterization of the plant community changes along the glacier foreland.

Composited soil samples were obtained from every second plot for a total of eleven samples along each transect at a depth of 5 cm to keep them consistent between recently-deglaciated areas and later successional areas (Dubuis et al. 2013). The soil samples were allowed to air dry for approximately one week between the time of collection and the time they could be transported to the laboratory facilities in University of Alberta, Edmonton. The soil samples were sieved through a 2mm sieve with the coarse fraction retained to determine the ratio of coarse to fine material. Samples were then stored in a 4°C cooler until analysis. Soil properties measured along the transects were: water fraction by volume (w.f.v), electrical conductivity (EC), pH, available nitrogen (ammonium, nitrate), carbon:nitrogen ratio (C:N), $\delta^{15}N$, and texture. Water content and EC were measured *in situ* with a Steven's HydraProbe Soil Sensor set to loam soils (default for soils with no *a priori* information). Moisture stress is not considered a likely constraint on plant life in these mountainous regions (Körner 2003), and the HydraProbe measurements confirm that assumption for our study sites in Bachelor Pass.

The pH, EC, NH_4^+ and NO_3^-, C:N, $\delta^{15}N$, and texture were all measured in the laboratory from the composited soil samples collected in the field. pH and EC measures consisted of mixing 10g of each soil sample with 20mL of water for a 2:1 water:soil ratio and determining the pH and EC of the resulting mixture with a calibrated Fisher AR 20 pH/EC meter. A 2 M KCl extraction was used to determine the amount of NH_4^+ and NO_3^- present in each sample. 50mL of KCl extractant was mixed with 5 g of air-dried soil per sample and shaken for 30 minutes. The resulting mixtures were then filtered and stored in a 4°C cooler until determination of NH_4^+ and NO_3^- concentration via colorimetric assay. For quantification of total nitrogen and total carbon to determine the C:N ratio as well as determination of $\delta^{15}N$, soils were finely ground to powder consistency using a ball grinder. Ground soil was then weighed into tin capsules, with less soil used for the isotope analysis. The soil properties were determined with a Costech 4010 Elemental Analyzer following the dry combustion method. Soil texture was determined using the hydrometer method. Samples were oven dried and mixed with 5% sodium hexametaphosphate and left for 24 hours. Samples were then mixed using an electric mixer and allowed to settle overnight. Samples were then mixed vigorously with a metal plunger for one minute to re-suspend the sediments after which a hydrometer was placed in the sample at the 40-seconds and 7-hours mark to determine the proportion (% by mass) of sand, silt, and clay in the sample. Samples that contained high carbonates had to be pre-treated with HCl prior to undergoing further sample processing.

Dendrochronological methods were used to determine the age structure and population density of subalpine fir (*Abies lasiocarpa*) along the primary transects. Trees were separated by height into two classes: individuals approximately 1 m height or above; referred to as >1 m, and individuals below 1m height but with an upright morphology (i.e. not krummholz); referred to as <1 m. Tree cores were collected from randomly selected >1 m subalpine fir trees in three different elevational bands (high, intermediate, and low terrain elevation, respectively). Tree cookies were also collected from randomly selected <1 m subalpine fir trees in each of the elevational bands. In total there were 5 individual fir trees selected of each size class in each elevational band for each transect, excepting the low elevational band which had 3 individuals selected of each size class in each transect due to time constraints. The age of each tree, both >1 m and <1 m, were determined by counting the annual rings in the tree core or cookie, respectively. For each focal tree identified, the distance to the closest ten nearest neighbours was measured as an estimator for density; excepting in the lowest elevational band where the closest 8 nearest neighbours were sampled and in situations where there were not ten individual subalpine fir trees within a reasonable distance. Nearest neighbour distances were used to determine the distribution of subalpine fir on the landscape. Average nearest neighbour distance was calculated and compared to an expected distribution based on the number of trees and size of the area of interest for each grouping (elevational band: high, mid, and low; transect: one or two; size class: >1 m or <1 m). The expected distribution is a theoretical random distribution pattern which corresponds to a ratio of observed over expected equals one. The random distribution is determined by:

$$\overline{D}_E = \frac{0.5}{\sqrt{n/A}} \qquad (1)$$

Where: n = sample size, A=area (m^2).

The ratio between observed and expected distribution determined whether the trees were in a clumped, random, or dispersed distribution pattern. If the nearest neighbour ratio is less than one the trees display a clumped distribution pattern, if the ratio is greater than one the trees are dispersed on the landscape. The relationship between tree age and terrain elevation was also investigated using a simple linear regression to determine an approximate rate of tree advance per year. By mapping this stochastic process in a linear fashion, we obtained an approximate value for the yearly rate of treeline advance over the past ~ 150 years. This value is associated with error both in terms of error associated with the spread of the data and with attempting to explain a stochastic process in a deterministic way. However, it is still useful to get an approximate picture of advance that has been occurring at this site.

Glacier recession was estimated using airphotos of the area from 1990-2005 and plotting the glacier margin on a topographic map obtained through GeoBC. ImageJ software was used to measure the distance between three key points and the glacier margin on the airphotos (Schneider et al. 2012). These values were then expressed as distance in meters and the difference in glacier margin over time was divided by the period of time the airphotos covered (15 years) to estimate the annual recession of the glacier. The method is an approximation to determine the yearly recession at the site to better understand how the upper boundary of the recently-deglaciated terrain is changing.

Statistical analyses

All multivariate statistical analyses and ordinations were run using R packages vegan and ecodist in R version 3.3.3 (Goslee and Urban 2007, Oksanen et al. 2017, R Core Development Team 2017). Non-metric multidimensional scaling (NMDS) ordinations were produced to visualize the differences between sites within each transect in terms of vegetation community. Each site was placed along the two NMDS axes, sites close together are more similar in vegetation composition, sites further away are more distinct. The soil physico-chemical variables were then overlayed onto the ordination to evaluate the contribution of each variable to the variation between the sites. Longer vectors correspond to stronger relationships between the soil variable and the differences between sites. Ellipses, corresponding to the three different elevational bands (high, mid, and low), were then added to the image to show the degree of overlap between sites that belonged to these categories. The more overlap between the ellipses the more similar each elevational band was to the others, i.e. identical communities at each elevational band would result in completely overlapping ellipses.

Canonical correspondence analysis (CCA) was used to evaluate how well the soil properties and topographic variables explain the variance in plant community composition. CCA determines synthetic gradients based on the community data and environmental variables to explain and ordinate the species data (ter Braak and Verdonschot 1995). The linear contributions of environmental variables to the ordination axes can then be used to explain the amount of variation in the species data (ter Braak and Verdonschot 1995). Soil properties used in the analysis were those remaining after removing multi-collinear variables identified by variance inflation factors (criterion: remove if $VIF > 4$). For transect 1 the remaining soil and topographic variables were: carbon:nitrogen ratio (C:N), $\delta^{15}N$, % clay, water fraction by volume, and slope. For transect 2 the remaining soil and topographic variables were: total carbon (TC), C:N, $\delta^{15}N$, % sand, water fraction by volume, and elevation.

Shannon-Wiener diversity index was calculated to determine α-diversity at each site as well as γ-diversity for the entire transect. The Shannon-Wiener H-index takes into account both species richness and evenness within a site to determine the diversity. α-diversity was also calculated using species richness and Simpson index (which is essentially a dominance calculation) separately. By calculating richness and dominance separately more complex relationships may be observed than by combining them into one metric (H-index). Dominance calculations were used to ensure trends in α-diversity using the H-index were robust and accurate (Morris et al. 2014). The Simpson index and species richness did differ compared to the H-index, and so the H-index was used for further discussion of the observed patterns. β-diversity was then calculated using Sørensen dissimilarity index which calculates the dissimilarity in species between each site. All diversity calculations were run using R package Vegan (Oksanen et al. 2017).

Pearson correlation matrices were built from the soil properties and topographic variables measured to identify collinearity and relationships between soil properties. These matrices were used to explore the data and understand the intricate nature of the soil parameters. VIF's were used to further identify and remove multi-collinear variables from all tests.

Results

Vegetation Communities

According to the NMDS ordination for transect 1, terrain elevation does not have a strong relationship with plant community composition (Figure A2.2). Each elevational band contained similar communities. The NMDS ordination for transect 2 does show a mild relationship between elevation and plant community composition (R^2= 0.523, p= 0.063) as sites within the same elevational band were clustered together (Figure A2.3). However, soil properties had stronger relationships between the sites for transect 1, specifically EC (electrical conductivity). EC was found to explain the most variation within the ordination (R^2= 0.714, p= 0.009). EC was not a strong predictor for transect 2 (R^2= 0.254, p= 0.332), and overall no variable was found to be significant for transect 2 ($p > 0.05$). $\delta^{15}N$, % clay, and elevation were the only variables ($p < 0.10$) showing a mild significant relationship with plant community composition in transect 2.

Canonical correspondence analysis (CCA) for transect 1 showed that soil properties (C:N, $\delta^{15}N$, % clay, H_2O fraction by volume, and slope; specifically) accounted for approximately 57% of the total inertia in the plant community composition across sites. CCA axis one accounted for approximately 39% of the constrained inertia, while CCA axis two accounted for approximately 28% of the constrained inertia. A permutation test was performed, with soil variables used in the CCA added sequentially. Only soil C:N ratio was found to be significant for explaining the community composition of transect 1 ($F_{1,5}$= 2.326, p= 0.006). CCA for transect 2 plant community composition data found that the soil properties included (TC, C:N, $\delta^{15}N$, % sand, H_2O fraction by volume, and elevation; specifically) explained approximately 76% of the total inertia for the community composition. CCA axis one accounted for approximately 34% of the constrained inertia while CCA axis two accounted for approximately 31% of the constrained inertia. The permutation test found that $\delta^{15}N$ was statistically significant for explaining the community composition ($F_{1,9}$= 2.621, p= 0.005). However, the total model itself was not significant for explaining community composition ($F_{6,9}$= 1.584, p= 0.081).

Vegetation cover of plant functional groups did not show a prominent pattern of species replacement along the elevational gradient for either transect (Figure 2.1, Figure 2.2). There was high variation in the percent cover of the functional groups for each elevational bin indicating that coverage changed considerably from plot to plot for each functional group along both transects. However, the one tree species found in the transects, *Abies lasiocarpa*, tended to be found only in the lowest elevational bins. *A. lasiocarpa* was found higher up in the second transect compared to the first. The other functional groups had considerable coverage differences between each 30 m elevational bin.

Lack of replacement generally corresponds to low β-diversity which was found for both transects. The Shannon-Wiener diversity index (H) were plotted to observe any trends in α-diversity along the elevational gradient. The first transect had the highest diversity near the higher and lower elevations of the transect, while the second had higher diversity in the mid elevations (Figure 2.3). There was high variation in α-diversity along the elevational gradient, which suggests that elevation is not the best predictor of species diversity. Other factors likely have a larger influence on the α-diversity of a given site and those factors change with along and between the transects. One likely property that effects the diversity of the sites is soil moisture which was different between the two transects due to topographical variation. This can have a large effect on community composition. Transect 1 has a more gradual consistent slope where transect 2 is "stepped" and holds less moisture as a result. This likely drives the soil moisture differences between the transects and may account for the diversity differences along the transects. Although soil moisture does not always limit plant growth in the alpine (Bowman et al. 1995), it still acts as a selection factor for what plant species will be able to thrive in different areas on a mountain (e.g. shrubs usually thrive in late-snowmelt areas compared to grasses).

Soil properties

Potential relationships amongst soil properties and topographic variables measured were explored using a correlation matrix (Table A2.1; typical values can be found Table 2.1). For transect 1, elevation was significantly correlated with both NH_4^+ and % silt ($r= 0.694$, $p= 0.017$ and $r= 0.603$, $p= 0.049$; respectively). NH_4^+ levels decreased downslope from the top of the transects and were comparatively low altogether. Similarly, % silt increased with elevation. NH_4^+ was mildly correlated with % silt as well where higher % silt coincided with higher values of NH_4^+. Slope was correlated with EC, pH, and C:N ($r= -0.862$, $p< 0.001$; $r= 0.715$, $p= 0.013$; $r= -0.615$, $p= 0.044$; respectively). Slope was negatively correlated with both EC and C:N, where steep slopes corresponded to lower EC and C:N values, but was positively correlated with pH. Steeper slopes were commonly found at higher elevations where there has been less time since deglaciation and lower vegetation coverage, and hence, these field locations would have experienced less weathering of parent material and soil formation processes. Concurrently, there was a negative correlation between pH and both EC and C:N ($r= -0.665$, $p= 0.026$ and $r= -0.660$, $p= 0.027$; respectively); however, EC and C:N were not significantly correlated with each other. This arrangement suggests a possible indirect connection between the two properties via their individual effects on pH. Collectively, these spatial relationships describe a gradient of soil development and indicate the result of complex vegetation feedbacks and time since deglaciation on developing soil properties and functions.

Elevation was significantly correlated with clay content ($r= -0.814$, $p= 0.004$), but no other variables in transect 2 (Table A2.1). Clay content was then also inversely correlated with soil NO_3^- ($r= -0.744$, $p= 0.014$), suggesting a possible indirect connection between elevation and NO_3^-, although it should be noted that all nitrate concentrations were very low (Table 2.1). Terrain slope was significantly negatively correlated with both TN and TC ($r= -0.900$, $p< 0.001$; $r= -0.873$, $p< 0.001$), as field locations with gentle slopes will tend to either build soil organic matter pools or experience lesser removals or erosion in part due to their implicit hydrology and vegetation development. As anticipated, TN and TC were also significantly positively correlated with each other ($r= 0.828$, $p= 0.003$).

Glacier extent

The rate of yearly recession was 11.9 m yr^{-1} according to airphotos obtained through GeoBC. The glacier margin moved a total distance of 179 m over fifteen years (from 1990-2005) (data not shown). It should be acknowledged that this airphoto-based approach for estimating glacial recession poses some implicit method uncertainty.

Treeline dynamics

The distance between the focal tree and its farthest nearest-neighbor was used as the diameter of the area calculation for the density estimate. All the trees regardless of grouping were found to be in a clumped distribution pattern (Table A2.2). For the >1 m trees, average age determined from cores decreased with increase in elevation, however there was no significant difference between the elevational bands or transects. Average age of <1 m trees did not change with elevation or between transects (Table 2.2). Age structure did not change significantly along the transects when comparing >1 m or <1 m trees ($F_{5,21}$:1.804, p=0.156 and $F_{5,20}$:1.058, p=0.412; respectively). However, the old:young ratio was different between the low elevational bands and the mid and high elevational bands for each transect (Table 2.2). The ratio ranged from 2.84 in the high band to 4.42 in the low band of transect 1 and from 2.89 in the high band to 6.73 in the low band of transect 2. The low band of transect 1 was not different from the mid and high bands of transect 2, however, the low band of transect 2 was higher than all other elevational bands. The old:young ratio shows that there is a small increase in <1 m trees (which are generally younger than >1 m trees) compared to >1 m trees in the higher elevational bands, but that the difference is slight. All of the <1 m were over ten years of age (average <1 m tree age was approximately 24 years) which, coupled with the ratio data, suggests that the treeline has not shifted upslope within the last ten years at least. The linear regression of mean total tree age and elevation determined an approximate rate of ecotone advance of 8 m yr^{-1} at our site during the last ~150 years, the period captured by our dendrochronological data. However, the regression was not significant (R^2=0.861, $F_{1,1}$=13.40, p=0.170). It is important to stress that this process of ecotone advance is not continuously occurring but is likely more stochastic in nature. This rate of tree advance provides an idea of the shift upslope that has occurred over time very likely due to the ameliorating climatic conditions above the treeline compared to as it was 150 years ago

(Figure A2.7).

Discussion

Topographic and edaphic drivers of plant community composition

Plant community composition was not strongly related to elevation within transects of 200 m elevational difference. Soil properties were generally more useful in explaining the variation between sites despite many earlier findings of elevation's importance on community structuring in mountain environments (Komárková and Webber 1978, Körner 2003, Boyce et al. 2005, Bruun et al. 2006), likely due to the local scale of our field transects. EC was found to be the only significant explanatory variable for community patterning in transect 1 according to NMDS ordination. This finding was somewhat surprising as pH was previously found to be one of the most important variables for explaining plant diversity and community composition models (Dubuis et al. 2013). EC can provide insight into the salinity, water content, and nutrient availability of the soil due to its connection to the amount and activity of available ions in the soil (Corwin and Lesch 2003). This suggests that the community composition differences are potentially related to differences in ion concentration in the soil solution, which may not be found by investigating parameters such as ammonium and nitrate concentrations directly, as EC gives a broader context of the amount of total ions and their activity which may be more stable in the soil temporally (Ryel et al. 1996, Lesch et al. 1998). In addition, since the EC measurements were low (less than 2 dS m^{-1}) the relationship between EC and plant communities is likely due to low ion concentrations from lack of nutrient availability primarily contributing to EC and not due to negative salinity effects (Rezaei and Gilkes 2005). EC is also related to slope catchment with higher EC values occurring on flat surfaces at the base of the catchment compared to high angled slopes which is another way EC may indirectly affect plant community (Rezaei and Gilkes

2005). pH is an indicator of the acidic-basic balance in the soil solution and hence pH and EC are interlinked. Soil pH has also been previously found to drive microbial diversity in alpine regions which indicates an additional indirect effect that pH has on plant species (Shen et al. 2013). Previous studies have also identified soil C:N ratio as being an important variable for predicting microbial community composition; as important as pH (Högberg et al. 2007, Shen et al. 2013).

The C:N ratio was found by CCA to have a strong relationship with plant community composition within transect 1, likely due to the association with the microbial community. The soil microbial community has reciprocal interactions with plant species and so can alter the plant community (Kardol et al. 2006). Soil microbes may have such a strong relationship with the plant community as they not only limit what plant species may grow in a location, but they are also changed by the plant species themselves. Many conifer species, such as fir and spruce acidify the soil around them which may facilitate some species while excluding others from an area (Cremer and Prietzel 2017). The primary tree species at our site were subalpine fir (*Abies lasiocarpa*) and Engelmann spruce (*Picea engelmannii*), both of which contribute to acidification (Cremer and Prietzel 2017). All plants contribute to litter deposition onto the soil which alters the C and N dynamics (Prescott 2002, Cremer and Prietzel 2017). $\delta^{15}N$, which was found to be mildly significant in transect 2, can act as an indicator of site age, nitrogen cycling, nitrification and mineralization dynamics, and topographic processes (Hogberg 1997, Chang and Handley 2000, Bedard-Haughn et al. 2003). $\delta^{15}N$ is affected by both long time-scale factors such as soil development and landscape topography and short time-scale factors such as nitrification and denitrification balance. In this way, $\delta^{15}N$ can indicate soil age and nitrogen process preference in an area, both of which are important for site selection by plant species.

For later successional species to advance and migrate upslope sufficient nutrients must be present in the soil to support their growth, as well as a suitable climate. Soils need to develop due to interactions with the current successional stage of the vegetation in an area in order to be amenable for the next successional stage. These plant-soil feedbacks are essential for succession to occur (Reynolds et al. 2003, Kardol et al. 2006, Van der Putten et al. 2013, Li et al. 2015). Facilitation by plant and microbes allows for the colonization of later successional plant species; which is the backbone of succession and soil development. It is through this process of plant-soil feedbacks that pH and EC are altered within an ecosystem, which gives them strong ties to the plant community.

Plant-soil feedbacks may be the driver of many edaphic variables measured including available NH_4^+, NO_3^-, and H_2O. The difference being that nutrient and moisture dynamics may change on shorter temporal scales (Ryel et al. 1996), and therefore be more responsible for within site diversity rather than between sites. Soil moisture is predictable on large-scales but is much more variable on short spatial scales (Oddershede et al. 2015). The spatial and temporal variation in moisture dynamics is thought to be a primary mechanism by which niche differentiation occurs for plant species (Araya et al. 2011). Since topography is tied to moisture, any differences in topography may allow for different sets of species to inhabit an area (Takahashi and Murayama 2014). The differences in the plant communities and diversity between transects likely reflects this relationship between topographical differences and niche availability.

Soil properties may be more important for plant community composition along a small meso-topographical gradient than elevation itself. At the local scale soil properties drive plant community composition, and a number of these properties will be affected by climate change. However, transect 2 had a slight association between elevation and plant community composition. There may be another factor that influences the strength of the relationship between elevation and plant communities at local scales. The stepped topography and its associated moisture dynamics of transect 2 may enhance the apparent effects of elevation compared to the more gradual terrain inclination of transect 1. The differing geomorphologies and associated hydrologies between ridges and depressions can have profound effects on how alpine plant communities are distinctively responding to climate change in temperate mountains.

Topographic and edaphic drivers of plant diversity

Bruun et al. (2006) found that local topography added significantly to models explaining species richness patterns in Fennoscandian mountains along with elevation. The topography was classified on a continuum from ridge to slope at a given elevation. The transects in this study did not cover as long an elevational range as Bruun et al. (2006), and so elevation may be a less prominent driver of diversity comparatively in our sites, however, our two transects were different from each other in terms of overall meso-topography. Transect 1 was located more in a depression formed by an active water-way and had higher average moisture content as a result, likely through high presence of snow in these areas. Transect 2 was located along a ridge line that contained multiple “steps” meaning that the elevational gain was punctuated by areas of relatively flat slope. These meso-topographical differences may explain much of the differences in diversity patterns between the two transects (Bruun et al. 2006, Takahashi and Murayama 2014). It also highlights the range of geomorphological variation that can be found within alpine landscapes, even just 200 m apart within the same slope aspect.

The α-diversity along the transects was low compared to previous research analyzing alpine plant diversity (Pierce et al. 2007). However, the β-diversity found between sites was consistent with previous research (Pierce et al. 2007), where β-diversity was low between sites generally due to the low regional species pool. The low γ-diversity is caused by the harsh abiotic conditions imposing strong filters on the diversity of vegetation species in alpine regions. Pierce et al. (2007) identified disturbance as the major driver of α-diversity in an alpine ecosystem by creating more available niche space for stress-tolerant and ruderal plant species. The authors also mention that the β-diversity is low in these areas due to stress and disturbance, in the end concluding that resource competition models may not accurately describe alpine regions as they do not account for disturbance and facilitation interactions. The β-diversity found at our site was also low, however it is difficult to determine with certainty whether that was due to disturbance or facilitation. As alpine areas are potentially subject to ground disturbance by squirrels and marmots, forest fires, rockfalls, and frost heaving, disturbance may play a role in the biodiversity of these areas.

The results of our cooccurrence analysis confirm previous findings that competition is less pervasive in alpine communities, whereas facilitation is more common (Figure A2.6; He et al. 2013, Mcintire and Fajardo 2014). We found mostly random cooccurrences between species; however, there were a few positive cooccurrences between species demonstrating that facilitation may be important in these areas as well. Previous work has determined that plant interactions are not a strong driver of community composition in subarctic-alpine areas (Mitchell et al. 2009), which may suggest disturbance as being the more likely candidate for the low β-diversity. Our study focused on the abiotic components determining community composition by acting as filters for α-diversity, however, future research into the role biotic relationships (inter and intra-specific, both between plant species and plant-microbe interactions) play in maintenance of plant community composition and how these relationships may be impacted by altered climate and edaphic variables due to climate change would be beneficial. Also, the relative importance of facilitation and disturbance in these areas influencing biodiversity patterns is important in building diversity models that are more suited to the unique conditions of alpine regions.

Soil variables along elevational transects

Elevation and slope were not consistently correlated with the same variables in both transects. There is variation in the strength of the relationship between these topographic variables and the soil properties measured. Some of this variation may be due to the interconnectedness of soil properties to each other. Properties such as soil texture will have a more profound influence on most of other soil properties such as moisture, organic carbon, and nitrogen mineralization (Jones 1983, Saxton et al. 1986, Burke et al. 1989, Hassink 1992, Silver et al. 2000). Elevation had a direct association with soil texture (i.e., proportion of fine particles) in both transects, either through the % silt or % clay contents of the soil. These texture measures then had indirect cascading effects on other soil variables, such as NH_4^+ and NO_3^- availability (Hassink 1992). Elevation may have indirect effects on soil properties that are, comparative to soil texture, acting on smaller time scales. Both elevation and texture remain relatively constant for a very long time, measured on geological time scales, whereas available nitrogen can change in a period of weeks or months depending on the weather, microbial community, and plant community in an area (Cain et al. 1999). This can result in variation in these properties across a relatively small spatial area. However, some of the more temporally constant soil properties, such as texture and pH (both of which change but more slowly than available nitrogen) may have stronger ties to topographic variables, which is evidenced by our results here. Elevation and slope may then correlate to smaller time scale variables, perhaps through their influence on soil texture.

Glacier extent

The Easy Glacier has been receding at an estimated rate of approximately 11.9 m yr^{-1} according to airphoto data that spanned fifteen years. The change in glacier margin over time will expose more recently-deglaciated terrain that plant species could potentially colonize. The newly-exposed terrain could allow alpine plant species to avoid the excluding effects of advancing treeline if they are able to survive under the harsh soil-weather conditions of the recently-deglaciated terrain. The rate of glacial retreat is a rough estimation, yet it provides an idea of how the landscape is changing recently in high, temperate mountainous regions and how these alpine environments will continue to change in the future.

Treeline dynamics

The distribution of tree species at all elevational bands was clumped according to the nearest neighbor density estimates. We expected that the distribution should change to dispersed as it gets closer to tree limit as there are fewer trees on the landscape. However, since the trees can only survive in suitable microhabitats that can support them above the treeline, it is possible that when these habitats are available, they will be filled with trees with the inhospitable area in between them devoid of trees. This pattern ensures that the spatial distribution of individuals does not change along the transect as they are still clumped within the areas that are amenable to growth. If these areas suitable for tree growth increase above the treeline then the species will start to expand into these areas which has been seen both above (treeline advance) and below (infilling) treeline (Körner 2003, Holtmeier and Broll 2007, Richardson and Friedland 2009, Camarero et al. 2017). The difference in old:young ratio between the low elevational band and the other two elevational bands suggest that the treeline may have advanced upslope as there are younger trees on average above treeline (low elevational band). However, since the youngest tree in the mid and high elevational bands was still over 10 years of age, it is not likely that the treeline has shifted much, if at all, in at least ten years at our transects. The evidence of potential treeline advance we found at our site suggests that the areas above treeline either became more amenable to tree growth over ten years ago, or that areas below treeline have a lower recruitment of seedlings, possibly due to overcrowding and competition. Since the difference between the age ratios is not very great in effect size and the youngest measured seedling above treeline was still over ten years old, it is difficult to say definitively that treeline is currently advancing in this area. However, the elevations above treeline do exhibit an increase in the proportion of trees <1 m in height in the population which suggests that these areas may be more amenable to recruitment which can lead to advance.

Previous studies have suggested potential threshold levels for climatic, topographic, and biotic properties controlling rapid colonization events for tree species near treeline (Bourgeron et al. 2015), therefore, conditions may have been adequate ~10-20 years ago at our study site and have since not passed all threshold conditions to allow another colonization event. The calculation of treeline advance over the past approximately 150 years shows that treeline has been advancing at a rate equivalent to 8 m yr^{-1}, presenting an approximation of how quickly areas above treeline are becoming adequate for trees to colonize due to ameliorating conditions. Further climate change could stimulate another rapid establishment period that would shift the current treeline upslope.

Across the treeline ecotone soil properties are most closely related to plant community composition which indicates them as being particularly important in structuring communities as climate continues to change. The magnitude and direction of change in soil properties, especially EC, C:N, and $\delta^{15}N$, will help determine the direction of change in plant communities along the gradient, and the change in plant community will have a reciprocal effect on soil properties. As treeline advances and glaciers retreat, the alpine community response will depend primarily on the soil properties along the gradient and the rate at which those key properties change.

Tables

Table 2.1. Average soil property values by zone for each transect with standard error. All analyses were conducted on composited soil samples collected for every second plot along each transect. Alpine zones for transects 1 and 2 only contained one value for each variable and so the variation is unknown, except for moisture as it was determined in situ for every plot in each transect. R-DG – Recently-deglaciated terrain; considered all of transect 3 (transect 3 moisture was averaged for all plots within the transect together).

Transect	Zone	pH	EC	NH4	NO3	TN	TC	C:N
			dS m^{-1}	mg kg^{-1}	mg kg^{-1}	mg mg^{-1} %	mg mg^{-1} %	
1	Montane	4.74±0.23	0.064±0.013	4.27±0.12	2.12±1.04	0.78±0.25	17.79±8.53	21.50±4.01
1	Subalpine	4.96±0.07	0.034±0.005	5.99±0.77	1.10±0.24	0.50±0.08	8.59±1.31	17.48±0.88
1	Alpine	5.09±NA	0.031±NA	10.03±NA	1.18±NA	0.25±NA	4.47±NA	18.25±NA
2	Montane	4.83±0.19	0.035±0.014	7.50±0.57	2.01±0.88	0.37±0.05	7.47±0.71	21.62±2.38
2	Subalpine	5.35±0.12	0.048±0.010	8.75±2.48	5.00±3.09	0.48±0.08	10.25±2.01	21.44±1.53
2	Alpine	5.42±NA	0.020±NA	24.74±NA	0.60±NA	0.29±NA	5.04±NA	17.31±NA
3	R-DG	7.92±0.12	0.091±0.018	10.11±2.14	1.05±0.26	0.01±0.01	0.44±0.11	79.88±25.64

Transect	Zone	δ^{15}N	% Clay	% Silt	% Sand	Moisture	elevation	slope
		‰				w.f.v	m a.s.l	°
1	Montane	5.63±0.91	13.0±1.5	42.7±7.2	44.3±6.1	0.49±0.05	1979±9.5	2.3±7.9
1	Subalpine	6.56±0.34	11.9±0.5	52.1±2.6	36.4±2.8	0.43±0.03	2076±16.3	14.6±2.4
1	Alpine	6.12±NA	14.0±NA	55.0±NA	31.0±NA	0.26±0.01	2146±NA	19.0±NA
2	Montane	6.60±0.66	11.0±0.2	45.8±4.1	43.0±4.5	0.33±0.06	2000±11.3	15.8±4.2
2	Subalpine	5.80±0.96	10.3±0.5	48.3±4.9	41.5±4.6	0.37±0.05	2081±13.2	13.5±5.0
2	Alpine	6.07±NA	10.0±NA	63.0±NA	28.0±NA	0.34±0.05	2136±NA	27.0±NA
3	R-DG	NA	16.4±2.6	44.4±9.8	39.4±8.5	0.17±0.04	2043±35.7	NA

Table 2.2. Average age (in years) for all trees, adults, and seedlings in each elevational band of both transects. Old:young ratio was calculated as the mean adult age of an elevational band divided by the mean seedling age in the same band. Old trees were >1 m in height and young trees were <1 m. The ratio shows more old trees compared to young at lower elevations likely due to the higher abundance of trees at the lower elevations compared to high elevations.

Transect	Elevational Band	Mean age	Mean age (>1 m)	Mean age (<1 m)	Old:Young ratio
1	High	41.1±8.1	60.8±10.0	21.4±1.9	2.84±0.57
1	Mid	57.7±11.9	83.3±14.9	27.0±3.7	3.09±0.75
1	Low	61.5±17.7	100.3±4.8	22.7±5.2	4.43±0.48
2	High	52.5±9.7	78.0±9.1	27.0±3.7	2.89±0.57
2	Mid	56.8±13.4	90.0±15.7	23.6±3.1	3.81±0.69
2	Low	67.0±23.0	116.7±13.2	17.3±0.3	6.73±0.23

Table 2.3. List of plant species recorded in transects 1 and 2 and their associated functional groupings.

Species	Functional Group
Abies lasiocarpa	Tree
Anaphalis margaritacea	Forb
Anemone drummondii	Forb
Anemone occidentalis	Forb
Anemone parviflora	Forb
Antennaria lanata	Forb
Arctostaphylos uva-ursi	Shrub
Arnica angustifolia	Forb
Arnica cordifolia	Forb
Arnica latifolia	Forb
Artemisia norvegica	Forb
Biological crust	Non-Vascular
Calamagrostis purpurascens	Graminoid
Carex capillaris	Graminoid
Cassiope mertensiana	Shrub
Castilleja miniata	Forb
Chamerion latifolium	Forb
Epilobium analgallidifolium	Forb
Equisetum variegatum	Graminoid
Erigeron grandiflorus	Forb
Erigeron peregrinus	Forb
Kalmia microphylla	Forb
Leptarrhena pyrolifolia	Forb
lichen	Non-Vascular
Luetkea pectinata	Forb
Maianthemum racemosum	Forb
moss	Non-Vascular
Parnassia kotzebuei	Forb
Petasites frigidus	Forb
Phyllodoce spp.	Shrub
Poa alpina	Graminoid
Polystichum lonchitis	Forb
Salix glauca	Shrub
Salix nivalis	Shrub
Salix vestita	Shrub
Saxifraga lyallii	Forb
Senecio triangularis	Forb
Sibbaldia procumbens	Forb
Vaccinium caespitosum	Shrub
Vaccinium scoparium	Shrub
Valeriana stichensis	Forb

Figures

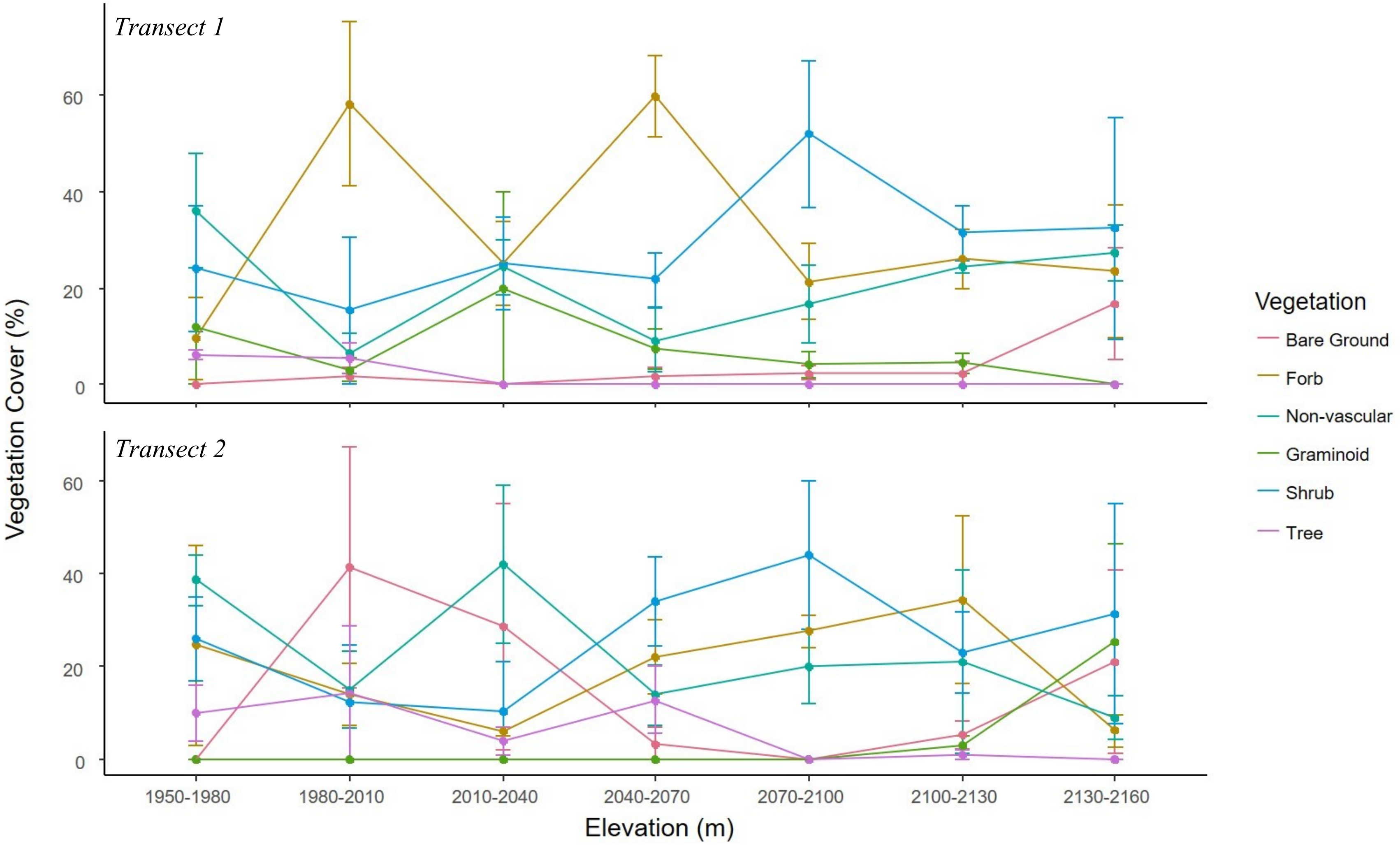

Figure 2.1. Percent vegetation cover of plant functional groups in 30 m bins along both transects. Plant species included in the groupings were chosen based on their prevalence in the sites and their contributions to % vegetation cover at a site. Rare plants that were at very few sites or contributed little to % cover were excluded.

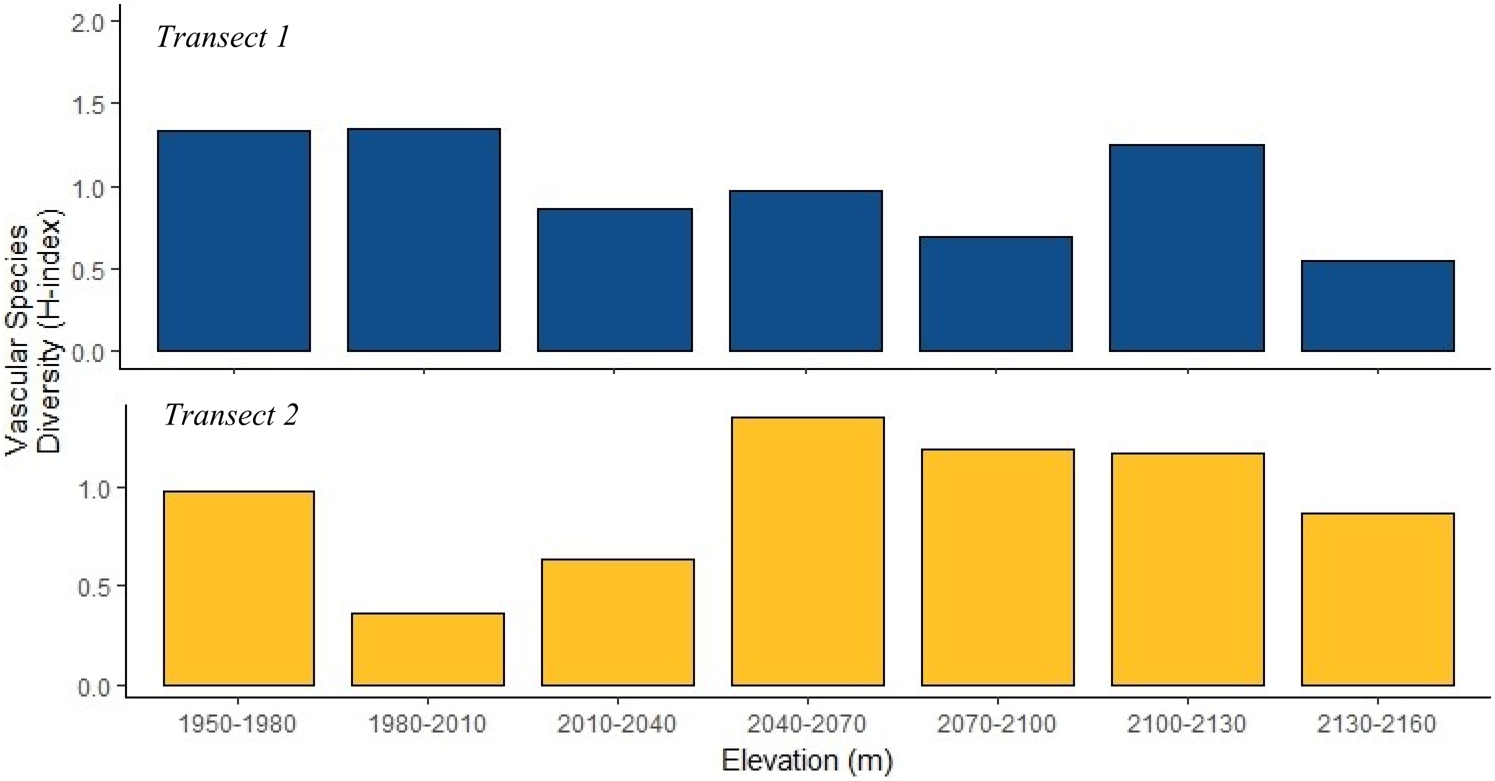

Figure 2.2. Shannon-Wiener Diversity H-index values in 30 m bins along both transects. The H-index was generally higher near the low end of transect 1, whereas for transect 2 the highest diversity values were in the middle of the transect. The diversity patterns differed between the two transects despite their close proximity, this is likely due to differences in the topography of the transects and how that relates to soil moisture and nutrient dynamics.

Appendix

Table A2.1. Correlation matrix between soil properties and topographic variables measured

	pH	EC	NH4	NO3	TN	TC	C:N	$\delta^{15}N$	% clay	% silt	% sand	elevation
EC	-0.665*	1				Transect 1						
NH4	0.481	-0.461	1									
NO3	-0.631*	0.645*	-0.352	1								
TN	-0.236	0.362	-0.395	-0.226	1							
TC	-0.455	0.394	-0.327	-0.069	0.920***	1						
C:N	-0.660*	0.390	0.019	0.280	0.316	0.640*	1					
$\delta^{15}N$	-0.136	-0.331	-0.264	-0.343	0.323	0.313	-0.093	1				
% clay	-0.283	0.204	0.197	-0.042	0.615*	0.706*	0.478	0.233	1			
% silt	0.371	-0.061	0.576·	-0.075	-0.547·	-0.635*	-0.290	-0.495	-0.376	1		
% sand	-0.326	0.008	-0.656*	0.078	0.462	0.526·	0.188	0.491	0.205	-0.983***	1	
elevation	0.539·	-0.597·	0.694*	-0.484	-0.552·	-0.520	-0.222	0.078	-0.285	0.603*	-0.591·	1
slope	0.715*	-0.862***	0.305	-0.511	-0.400	-0.496	-0.615*	0.375	-0.360	0.034	0.040	0.578·
EC	0.225	1				Transect 2						
NH4	0.390	-0.293	1									
NO3	0.115	-0.131	-0.349	1								
TN	0.319	0.348	-0.345	0.400	1							
TC	0.071	0.150	-0.407	0.711*	0.828**	1						
C:N	-0.526	-0.324	-0.290	0.479	-0.238	0.327	1					
$\delta^{15}N$	-0.204	0.348	-0.434	0.225	0.033	-0.043	-0.119	1				
% clay	-0.256	0.475	-0.112	-0.744*	-0.359	-0.506	-0.130	0.019	1			
% silt	0.027	0.245	0.232	0.015	-0.086	-0.189	-0.344	0.425	-0.213	1		
% sand	0.023	-0.323	-0.191	0.055	0.119	0.231	0.347	-0.442	0.095	-0.992***	1	
elevation	0.559·	-0.289	0.408	0.440	0.274	0.287	-0.120	-0.045	-0.814**	0.224	-0.108	1
slope	0.036	-0.198	0.481	-0.504	-0.900***	-0.873***	-0.020	-0.133	0.371	0.147	-0.171	-0.102

· $0.1<p<0.05$, * $0.05<p<0.01$, ** $0.01<p<0.001$, *** $p<0.001$

Table A2.2. Average and standard error density data of subalpine fir in Bachelor Pass separated by age class, transect, and elevational band. Radius was determined as the distance between the furthest two trees of the same age class, transect, and elevational band and was used to determine the area for the trees of those classes. Expected mean distance to nearest neighbour (D_E) was determined from the number of trees measured and the area in which they were measured. Average Nearest Neighbour ratio (ANN) was determined as the observed mean distance between nearest neighbours (D_O) divided by the expected, which corresponds to a random distribution. If ANN < 1 the trees follow a clumped distribution pattern. Standard error of the mean for Area and D_E for adult and seedlings separately are unknown as they are equal for all individuals within a single transect and elevational band.

Size class	Transect	Elevational Band	Area	D_O	D_E	ANN
			m^2	m	m	
Total	1	High	33646.07±1509.82	3.91±0.49	13.07±0.25	0.3±0.04
Total	1	Mid	24426.68±3336.13	3.09±0.47	10.2±0.63	0.31±0.05
Total	1	Low	9538.61±2222.54	1.46±0.29	9.6±1.21	0.15±0.02
Total	2	High	80158.45±3550.94	2.86±0.31	19.98±0.44	0.14±0.02
Total	2	Mid	13120.1±748.74	2.51±0.34	8.21±0.28	0.31±0.05
Total	2	Low	15787.55±101.64	1.16±0.19	12.6±0.04	0.09±0.01
> 1 m	1	High	38175.52	3.35±0.85	13.82	0.24±0.06
> 1 m	1	Mid	34057.27	3.12±0.84	12.01	0.26±0.07
> 1 m	1	Low	14508.36	1.98±0.37	12.29	0.16±0.03
> 1 m	2	High	69505.64	2.56±0.52	18.64	0.14±0.03
> 1 m	2	Mid	10873.88	2.64±0.64	7.37	0.36±0.09
> 1 m	2	Low	15560.29	1.57±0.05	12.73	0.12±0.004
< 1 m	1	High	29116.61	4.47±0.42	12.31	0.36±0.03
< 1 m	1	Mid	12869.97	3.06±0.39	8.02	0.38±0.05
< 1 m	1	Low	4568.87	0.95±0.12	6.90	0.14±0.02
< 1 m	2	High	90811.26	3.17±0.33	21.31	0.15±0.02
< 1 m	2	Mid	15366.33	2.39±0.33	9.04	0.26±0.04
< 1 m	2	Low	16014.82	0.74±0.04	12.92	0.06±0.003

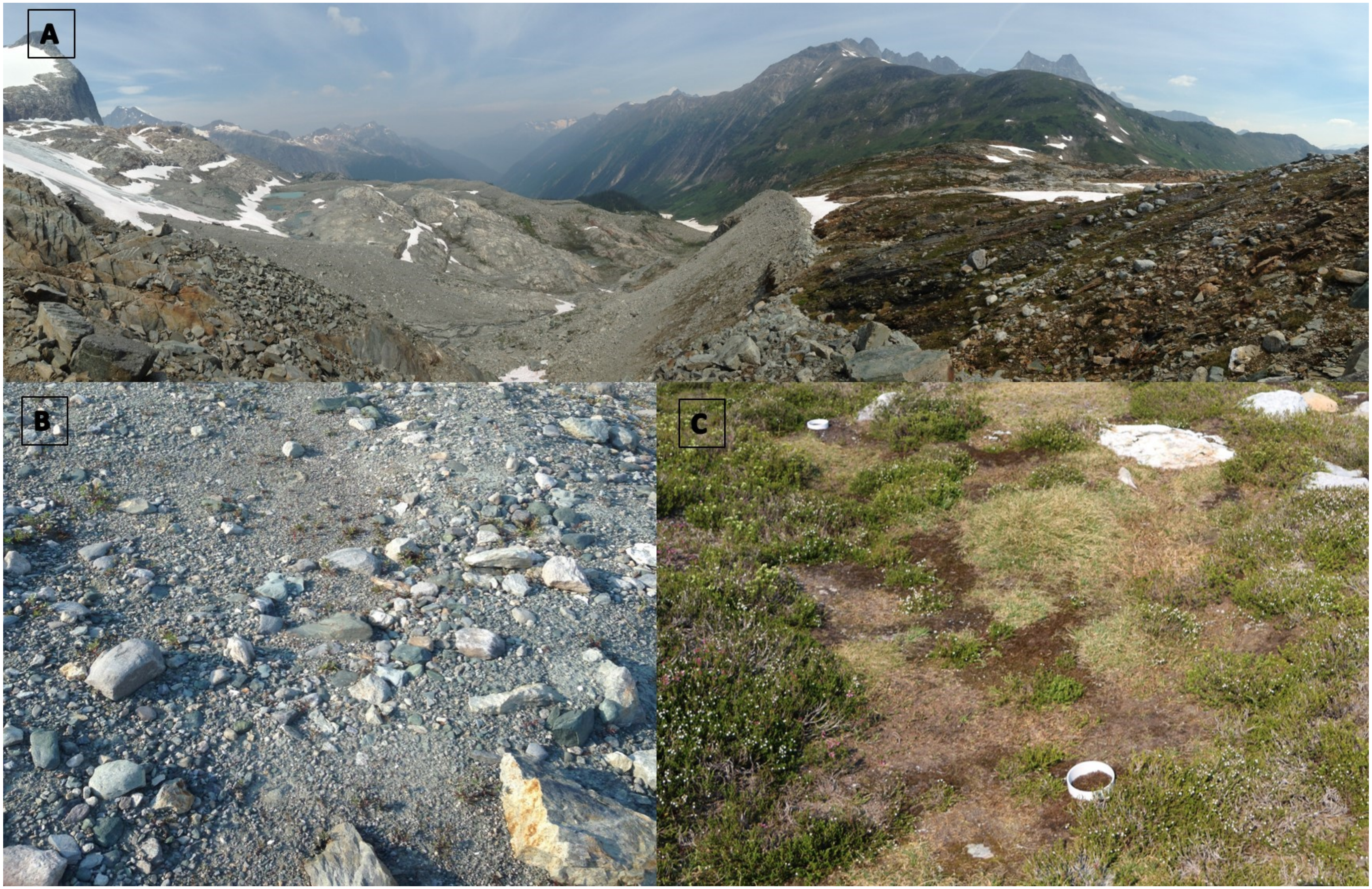

Figure A2.1. Site photos for RDG (B) and alpine (C) sites. Panel A shows both site types and the amount of separation between them.

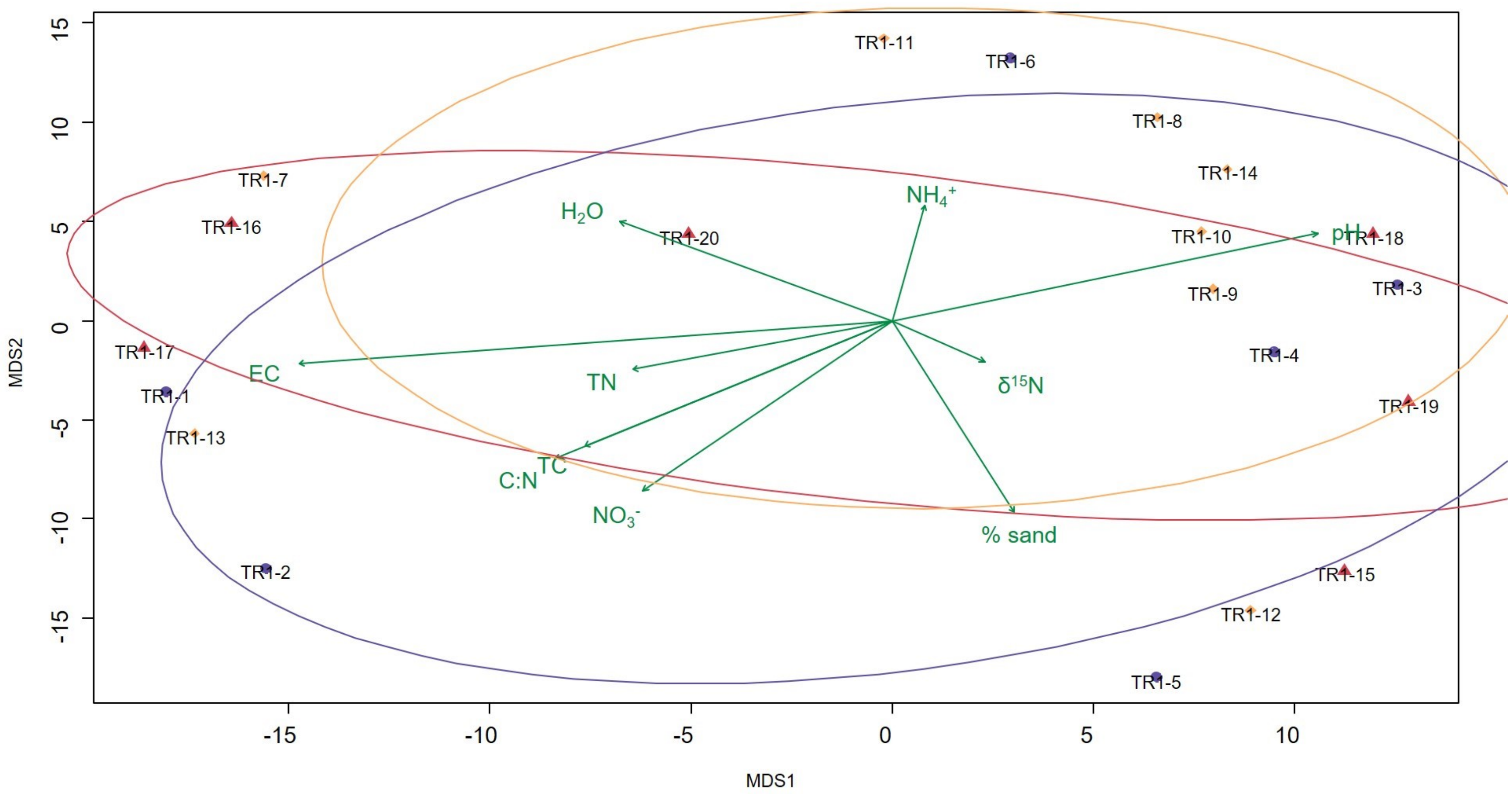

Figure A2.2. NMDS ordination of the transect 1 sites. Plant community composition at each site did not group according to elevation but are rather driven by soil properties, namely electrical conductivity (EC). Vectors represent the soil properties measured and their contribution to the variation between sites. Ellipses correspond to the different elevational bands: low band = blue, mid band = yellow, and high band = red.

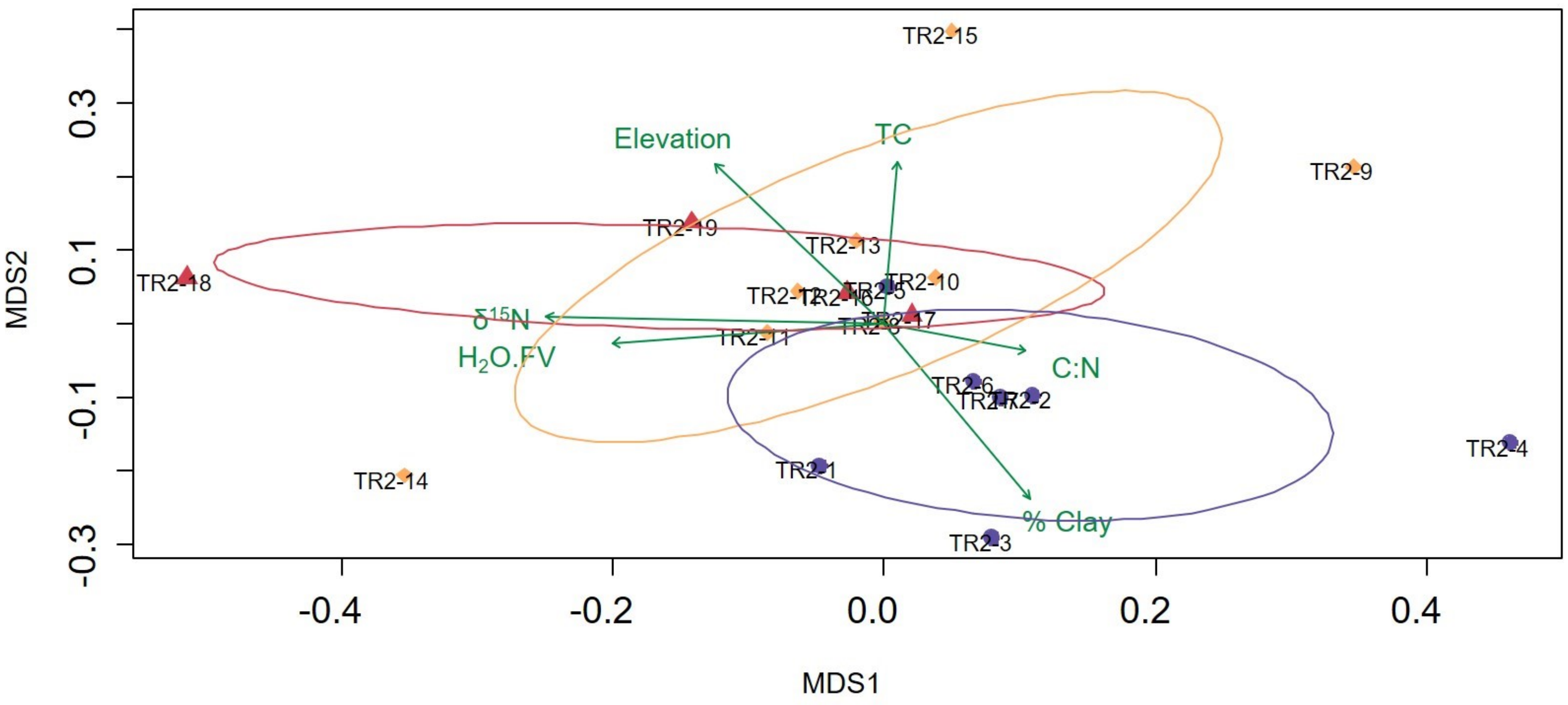

Figure A2.3. NMDS ordination of the transect 2 sites. Plant community composition at each site was somewhat grouped into their elevational bands, however there is still overlap between the groupings. There were no significant variables for explaining plant community composition in transect 2, however, $\delta^{15}N$, % clay, and elevation were mildly significant at an alpha of 0.1. Vectors were added representing the soil properties measured and their contribution to the variation between sites. Ellipses correspond to the different elevational bands: low band = blue, mid band = yellow, and high band = red.

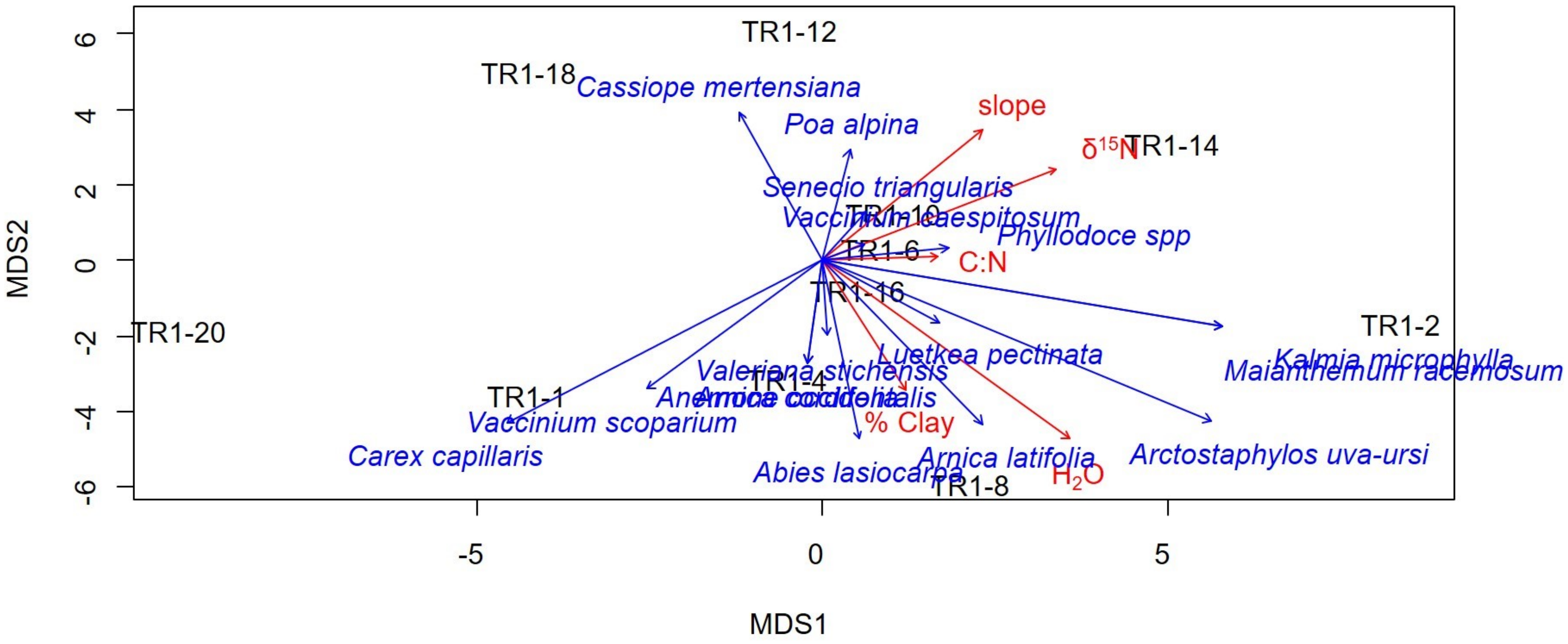

Figure A2.4

. Direct gradient analysis of plant species and soil properties using NMDS ordination of transect 1 data. The length of the vector corresponds to the strength of the association.

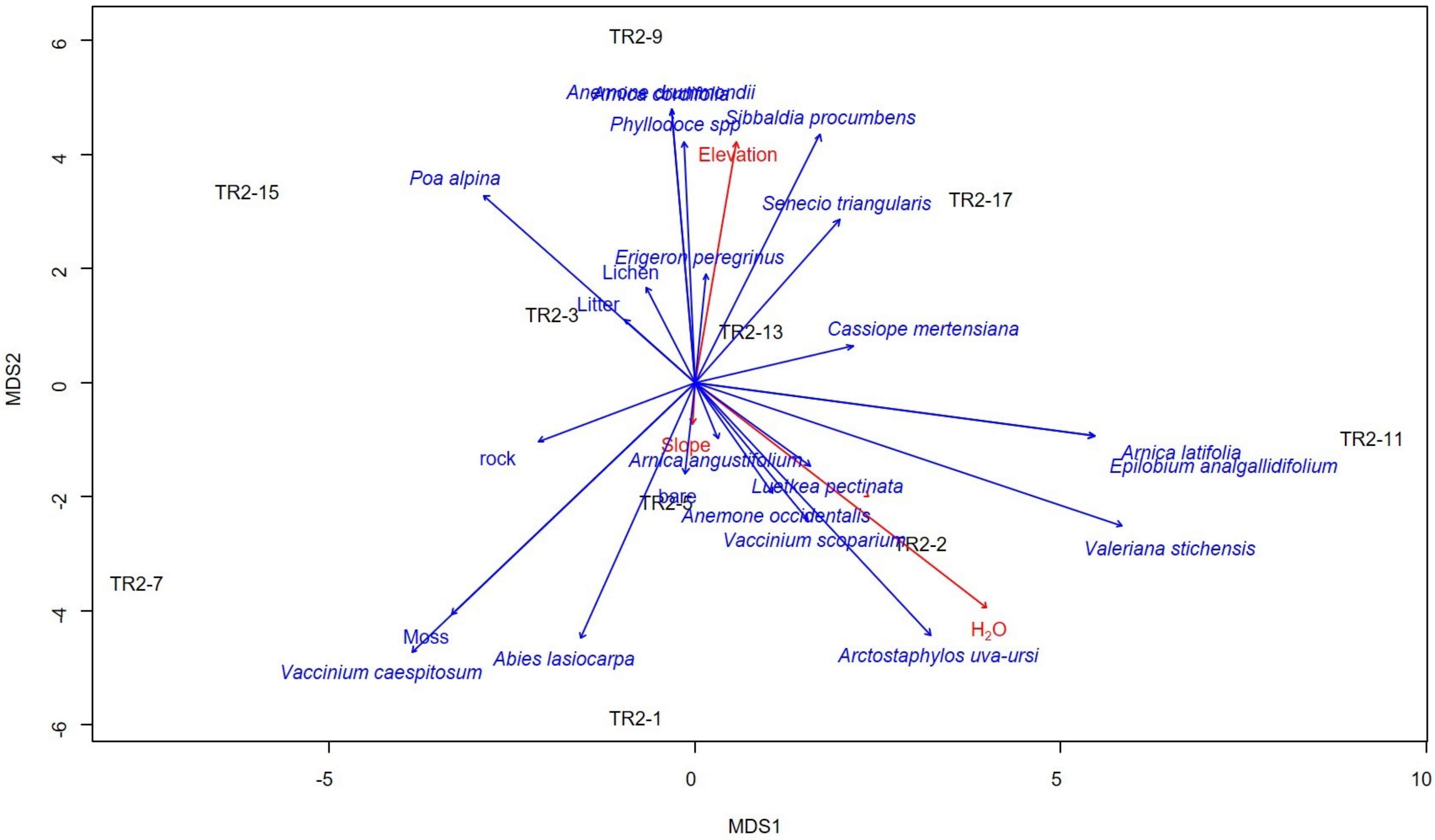

Figure A2.5. Direct gradient analysis of plant species and soil properties using NMDS ordination of transect 2 data. The length of the vector corresponds to the strength of the association.

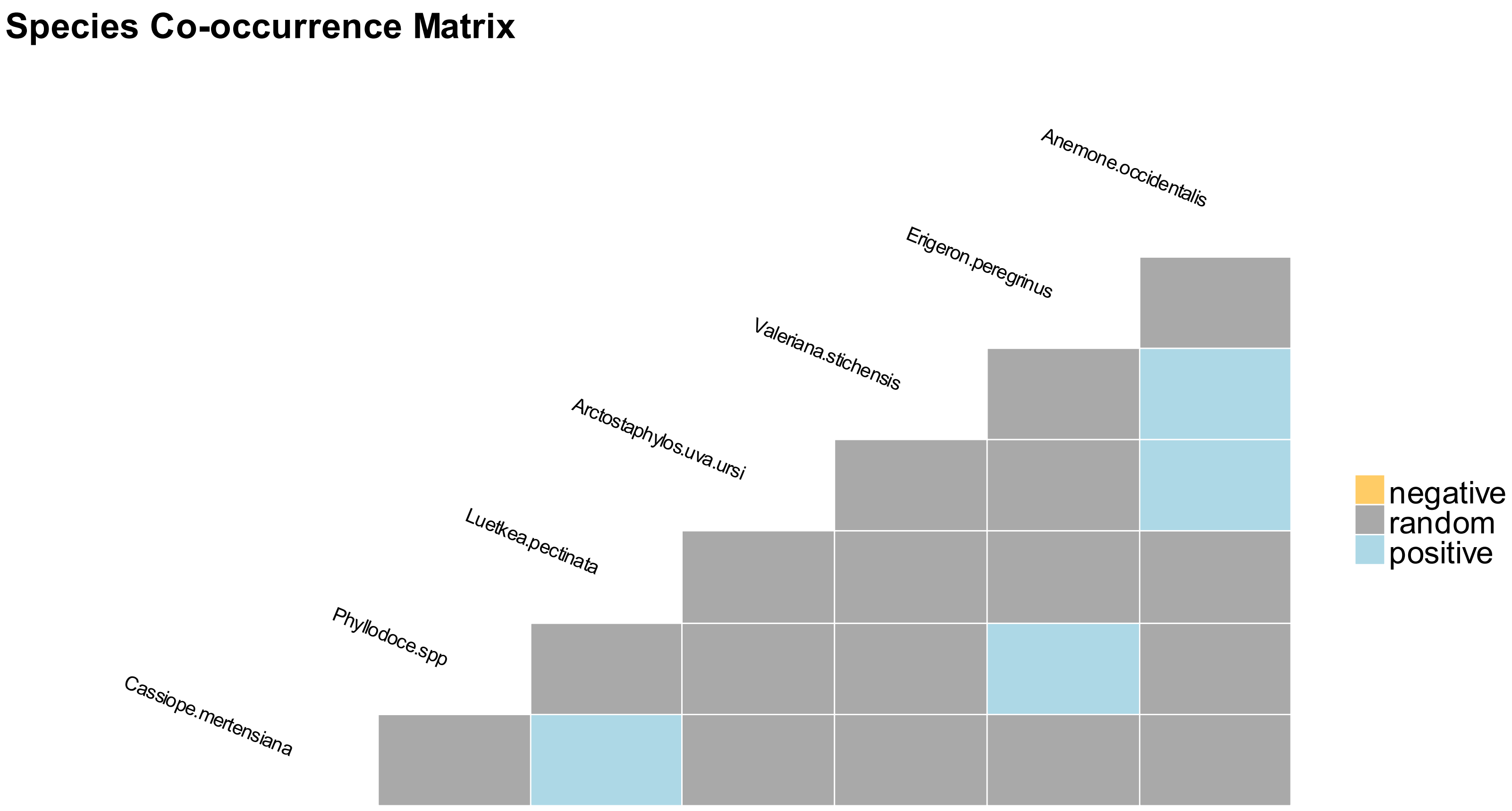

Figure A2.6. Instances of cooccurrence between species along transect 2. Most of the interactions between species were random, however there were four instances of significant cooccurrence between species indicating potential facilitation interactions. Competitive interactions were not found at all. Negative associations indicate competition, positive associations indicate facilitation.

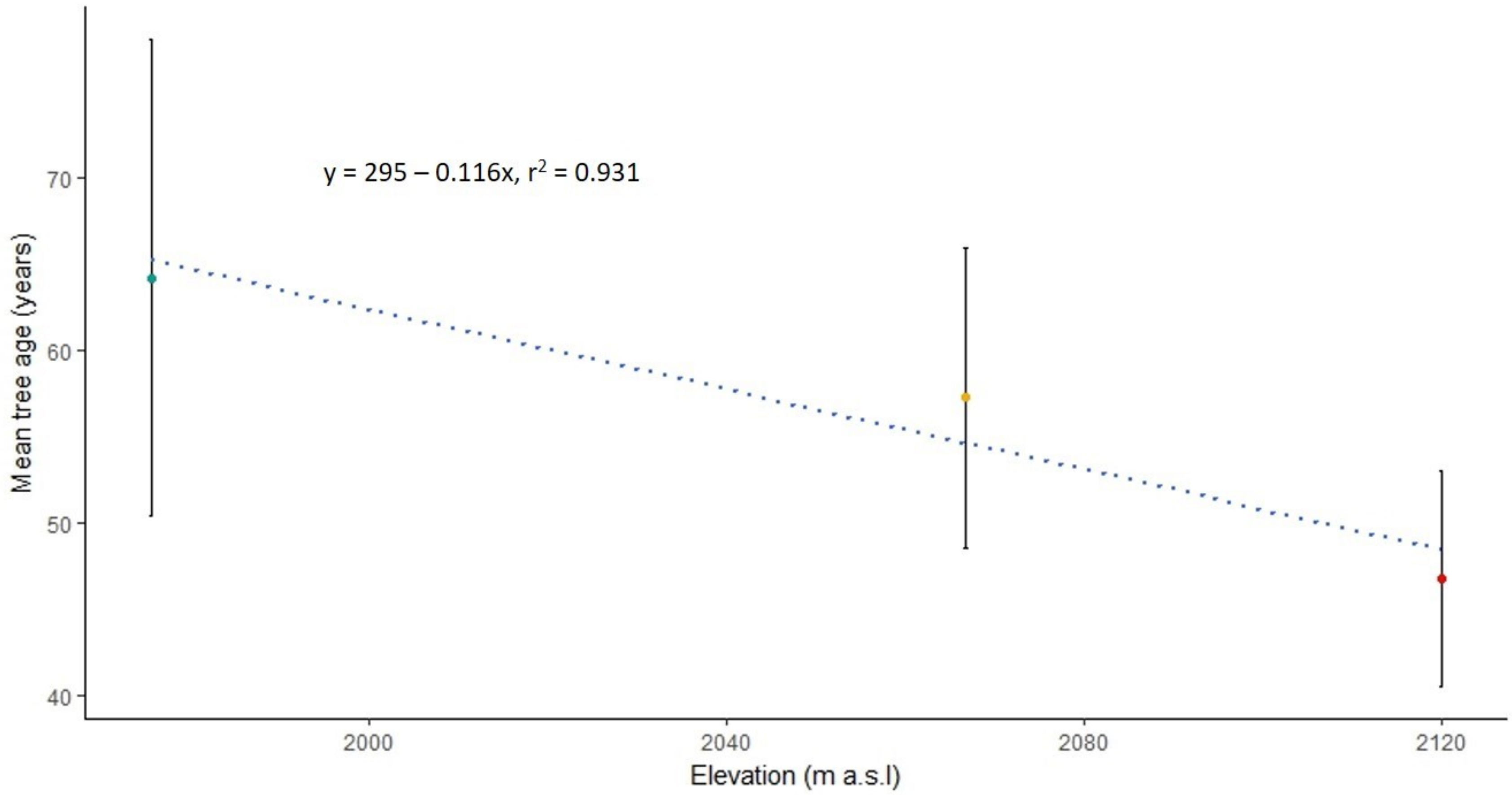

Figure A2.7. Average total tree age in years compared to the elevation gain associated with each elevational band. The average elevation of the low band was set to zero and the gain in elevation associated with the average of the mid and high bands was then determined. By comparing the average age of the trees in each band to the elevation gain using a regression the average rate of advance of treeline was determined to be approximately 8 m yr^{-1}. The line corresponds to the regression line where the absolute value of the slope is equal to the rate of advancement. Error bars represent the standard error of the mean tree ages for each band.

Chapter 3

Differences in soil properties does not limit growth and survival of alpine plant species in recently-deglaciated terrain

Introduction

Climate change has led to changes in the spatial distribution of species (Klanderud and Birks 2003, Walther et al. 2005, Corlett and Westcott 2013, Alatalo et al. 2016). The expansion of dominant species into new ecosystems can have adverse effects on the local biodiversity often mediated by competition (Corlett and Westcott 2013). The potential for a plant species adapted to harsh environmental conditions to expand into newly available territory may determine its likelihood of survival. Conversely, species may also respond by adapting to local conditions as they change, including both competition with new species and tolerance of changing abiotic conditions.

Alpine regions are an ideal place to test the ability of plant species to react to climate change by range expansion. Climate warming occurs in the alpine at an accelerated rate compared to lowland regions and treeline expansion is widely occurring in many alpine habitats (Spasojevic et al. 2013, Brown et al. 2014). The upslope expansion of the treeline has the potential to compete with or displace current alpine vegetation at these elevations. However, soil conditions, growing season temperature, and winter snow cover have been identified as limiting the advance of current treelines in Canada (Davis and Gedalof 2018). Current soil and temperature conditions may be unsuitable for tree species allowing shrubs and herbaceous vegetation a longer timeframe than initially believed to respond to changing climate conditions. The rate of soil development may be rate limiting for both treeline advance and alpine vegetation establishment in recently-deglaciated terrain.

Concurrently glaciers are receding in alpine regions exposing new terrain for species to inhabit (Barry 2006, Fischer et al. 2014, Lane et al. 2017). It is not well known if alpine-adapted plants will be able to expand their range into more recently-deglaciated terrain as climate warms and treeline advances. Recently-deglaciated terrain is largely devoid of nutrients and has a limited microbial community which prevents most species from being able to colonize it. With ongoing anthropogenic activities, namely burning of fossil fuels, the rate of nitrogen deposition/fixation from the atmosphere is expected to increase (Galloway et al. 1994, Bowman et al. 1995, Zavaleta et al. 2003), which may speed up the rate of soil development and primary succession in recently-deglaciated terrain, potentially allowing later-successional alpine species to colonize it earlier.

Nitrogen is an often-limiting nutrient in recently-deglaciated terrain that plants require to photosynthesize and grow (Bowman et al. 1995, Schmidt et al. 2016). Early-colonizing plants are typically well suited to dealing with nutrient deficient soil conditions which allows them to expand into areas that non-colonizers cannot (Chapin 1993). Later successional plants (referring to any plants that are a part of seral stages after initial colonization) depend on nutrient pools being built up over time by deposition and plant litter decomposition before they can expand into these areas (Connell and Slatyer 1977, Jones and del Moral 2009). Increasing the amount of nitrogen in the system should allow species that were previously unable to survive in the area to grow and establish. Increasing nutrient availability may have a drastic effect on the plant community during early succession.

Alpine ecosystems are primarily composed of herbaceous vegetation with some low-lying shrubs such as heather. There is potential for alpine vegetation to become threatened as treeline advances if they are not able to expand or migrate upslope into new territory. Recently-deglaciated terrain exposed by glacial recession may act as refugia for alpine vegetation if the plants are able to grow and survive in this nutrient depleted terrain. Specific alpine species are likely better able to expand into these areas than others based on their adaptations for growing in these adverse conditions. As well, altered edaphic variables due to climate change, such as increased soil temperature, soil moisture and nitrogen levels, may allow later-successional alpine species to establish in recently-deglaciated terrain. The ability for alpine vegetation to survive in recently-deglaciated terrain is not well understood under current or amended soil conditions. Transplantation of alpine species into recently-deglaciated terrain is one experimental approach to determine how focal species may be impacted by the soil conditions in recently-deglaciated terrain.

Our primary objective was to assess the ability of four focal later-successional alpine species to grow and survive in recently-deglaciated terrain, and to determine if increased nitrogen in the soil would increase growth or survival of these species. We specifically targeted two graminoids and two forbs as they were characteristic of species most likely to move into these areas based on current successional patterns (Jones et al. 2003, Tscherko et al. 2005). We predicted that plant growth and survival of these later-successional species would be low in recently-deglaciated terrain but that the species would still be able to survive into the following growing season. We predicted that a nitrogen addition will increase the growth and survival of the focal species, though the increased growth from additional nitrogen may not be observable in the original season of application due to factors such as nitrogen immobilization by soil microbes (Körner 2003). The graminoid species specifically will benefit from the additional nitrogen which will increase productivity, measured via growth rate (Farrer et al. 2016).

Methods

Study site

The study was conducted at Bachelor Pass (51° 31'21.06"N; 117° 57'31.55" W), in the Northern Selkirks, Columbia Mountains, British Columbia. The experiment was conducted in the 2016 growing season (late June to late August) and over-winter data gathered in early August 2017. The vegetation of the site was composed of typical alpine heath-shrub and grasslands in the pass (referred to as alpine), and primarily of dwarf fireweed and other colonizer species in the recently-deglaciated terrain. Soils in the alpine sites were typically Brunisols and soils in the recently-deglaciated sites were Regosols.

Transplant methods

Five focal plant species were selected for the transplant experiment, four later-successional species and one early-successional species. These are: leather-leaved saxifrage (*Leptarrhena pyrolifolia*; Saxifrage hereafter), alpine bluegrass (*Poa alpina*; Poa hereafter), lesser blackscale sedge (*Carex atrosquama*; Carex hereafter), and Norway sagewort (*Artemisia norvegica*; Artemisia hereafter) classified as later-successional species, and dwarf fireweed (*Chamerion latifolium*; Chamerion hereafter) classified as an early-successional species and used as a control to determine how species adapted to recently-deglaciated soils are affected by transplantation and nitrogen addition.

Leptarrhena pyrolifolia is a small forb with alternate, leathery leaves that are deep green on the upper surface and brownish beneath. The rootstock is woody and horizontal and the seeds are dry and winged (Soltis 2016). The plants are typically found on moist slopes and stream banks from the subalpine to alpine (Moss and Packer 1994). *Poa alpina* is an erect graminoid generally 10-30 cm tall. The leaves are on short basal shoots and the blades are also short. Poa spikelets are purplish to bronze with ovate glumes, their seeds are also winged indicating wind dispersal (Tackenberg and Stöcklin 2008). *Poa alpina* are found in subalpine to alpine meadows and rocky surfaces (Moss and Packer 1994). *Carex atrosquama* is a sedge generally 20-50 cm tall with dried leaves from previous years obvious at the base of the plant. The leaves have well developed blades and the spikes are purplish-black. *Carex atrosquama* seeds are not well studied, however, alpine Carex species have been found to be primarily wind dispersed as an adaptation to the environment (Newhouse et al. 1995). They are primarily found in subalpine meadows (Moss and Packer 1994). *Artemisia norvegica* is a small perennial forb with a stout woody rhizome. Lower leaves are twice-pinnately dissected, whereas the upper leaves are less dissected. Flowering heads are large and nodding with blackish margins. Artemisia species are typically sand or wind dispersed, though, the dispersal mechanism for *Artemisia norvegica* is not explicitly known (Ma et al. 2010). These plants are typically found on subalpine and alpine slopes (Moss and Packer 1994). *Chamerion latifolium* is a small perennial forb that form colonies. Their leaves are broadly lanceolate to elliptic, and their inflorescence is a raceme with pink to rose-purple petals. Their seeds are small, attenuate, and easily dispersed (Sharpe et al. 2008). *Chamerion latifolium* can be found primarily on gravel bars, stream banks, and scree slopes up into the alpine (Moss and Packer 1994).

The collection sites for the later-successional species were at the eastern edge of the pass (Artemisia and Carex), at the base of Pyrite ridge overlooking the west valley (Poa), and further down the glacier foreland from the recently-deglaciated sites (Saxifrage). The transplant sites spanned across the pass and in the recently-deglaciated terrain below the Easy glacier (Figure A3.1). The growth and survival of focal individuals were determined using appropriate measures for each species over the course of the growth season. Variables measured include height, number of leaves, number of flowers, longest leaf length, and stem diameter. Plants were re-measured in the following field season to determine survival and between season growth (Alexander et al. 2015). In August 2017 all remaining individuals were collected and the dry weight of above and belowground biomass measured to determine allocation of resources within the plant.

Four sites were classified as recently-deglaciated (referred to as transplant sites) and were the sites of transplantation of the focal species. These sites were located close to the Easy Glacier, and were determined based on proximity to each other, apparent condition of the soil, and similarity in plant composition. A total of forty individuals of each focal species were moved from their respective alpine grassland sites in Bachelor Pass (referred to as alpine sites) into the transplant sites; ten individuals per transplant site. Forty individuals of each focal species were also identified within their original alpine sites as a control, with twenty additional individuals per species transplanted within their original site (referred to as within-site transplants) to control for the effect of transplantation on growth and survival (Hu et al. 2016). Eighty individuals were identified for the control species and categorized into transplant (within site) or control (not transplanted) individuals. Each transplant site therefore had twenty Chamerion individuals with ten being transplant and ten being control individuals (Table A3.1). Transplanted individuals of each species were moved with some of the original soil remaining around the roots to avoid damaging the fine roots which could be a detriment to growth and survival.

Half of the transplanted individuals for each of the five focal species were supplemented with inorganic nitrogen in the form of ammonium nitrate at a rate of 20 kg N ha^{-1} yr^{-1}. The rate of supplementation is 2.8x higher than the estimated natural nitrogen deposition in the Colorado

Rocky Mountains (i.e., 7 kg N ha^{-1} yr^{-1}) as determined by Burns (2003). The amount of added nitrogen was large relative to natural levels, but still within potential limits of increasing nitrogen deposition due to climate change (Körner 2003). Plants received the full 20 kg N ha^{-1} yr^{-1} nitrogen addition twice, once after transplantation and once at the end of the first growth season; the total nitrogen addition for the over-winter period was therefore 40 kg N ha^{-1} yr^{-1}. Growing season growth and survival of nitrogen added plants is due to the first nitrogen addition while those that survived over the summer received the additional nitrogen supplementation for over-winter. The ammonium nitrate was dissolved in water at a concentration of 0.03 g N mL^{-1} H_2O, and each plant received a volume of approximately 40 mL. Zero nitrogen control individuals received an equal amount of water instead of ammonium nitrate fertilizer to account for moisture differences between treatments. In 2017 the biomass samples collected from the field were ground and used to determine the nitrogen concentration of the above and belowground biomass.

Growth rate of focal plant species was determined as the difference in size of growth metric (such as: longest leaf, height, diameter, number of leaves, etc) between two growth intervals. Each growth interval was two weeks starting in the week of July 11, 2016, with three growth intervals total, meaning growth and survival was measured over six weeks during the growing season 2016. Mortality was considered to have occurred when there was no more green tissue and the plant was completely desiccated.

Soil properties

Composited soil samples were collected for each transplant and alpine site in both the 2016 and 2017 seasons. The physico-chemical properties measured were: water fraction by volume, electrical conductivity (EC), pH, available nitrogen (ammonium, nitrate), carbon:nitrogen ratio (C:N), $\delta^{15}N$, $\delta^{13}C$ (in the 2017 growing season only), and texture (in the

2016 growing season only). The properties measured allow for estimation of potential limiting factors in plant growth and survival, and how that may change over the course of a year. Soil surface and 5cm belowground temperature was also measured using HOBO data loggers placed in the RDG, saxifrage (location of *Leptarrhena pyrolifolia* origins, downslope from the RDG sites and not in the pass itself), and alpine sites. The difference between the surface and belowground probes allows for determination of the insulative capacity of the soil to heat transfer in the recently-deglaciated and later successional areas (Sklenár et al. 2016). Eighteen additional composited soil samples were also collected using ethanol sterilized tools and whirlpaks to be used in a phospholipid fatty acid analysis to determine the changes in microbial community, specifically the bacteria:fungi ratio, between the transplant, RDG (in recently-deglaciated terrain, but not the site of transplantation), and alpine sites in the 2017 season, with 6 samples being collected for each site type. Soil samples for microbial analysis were kept on ice in a cooler for less than 48hours total before being transferred to a -80°C freezer at the University of Alberta. Following extraction of the PLFA's, samples were analyzed using gas chromatography (GC) to determine the PLFA's present in each sample.

Data analysis

The difference in growth rate between the control, transplant, and within-site transplants as well as between the nitrogen addition and non-nitrogen-added plants was determined by a series of student's t-tests between the groups. Survival between control, transplant, and within site transplant, as well as between control and nitrogen added plants was determined by proportion tests which use a χ^2 distribution to determine the difference between the proportion of one categorical or ordinal variable (survival) compared to the proportion of another group determined by another categorical variable (in this case site or nitrogen addition).

Bacteria:fungi (bac:fun) ratio was determined by identifying various bacterial and fungal marker PLFA's (Frostegård et al. 1993, 2011, Frostegard et al. 1996, Högberg et al. 2007, Hahn and Quideau 2013) present in the soil samples collected and then determining the amount of PLFA's of each marker present for each site. Afterward the bacterial and fungal PLFA were compared using a ratio. The bac:fun ratio was compared across site types (transplant, RDG, and alpine) using a one-way ANOVA as well as a series of t-tests. Comparisons of the bacterial or fungal PLFA's between sites individually was accomplished with a one-way ANOVA and a series of t-tests as well. Finally, comparison of the amount of gram negative compared to gram positive bacterial PLFA's was performed using t-tests for each site type.

Results

Growth

Longest leaf metrics were chosen as the growth metric for all focal species since they have the most complete record and highest variability over time. In addition, graminoids are known to compensate most rapidly to nutrient additions by elongation of their leaves (Chapin 1980).

Transplanting had a significant negative effect on Artemisia ($t_{68.265}$=2.657, p=0.010), Carex ($t_{54.369}$=3.227, p=0.002), and Chamerion ($t_{77.993}$=3.157, p=0.002) longest leaf growth rate for the first growth interval. However, there was no difference in the other two intervals which shows that, of the plants that survived, the growth rate was comparable to control plants (Figure 3.1). Poa blade length growth rate was significantly different between transplants and control plants in the second interval ($t_{69.642}$=-2.035, p=0.046), but none others. This may be a delayed effect of transplanting on growth rate which recovered in the third interval. Transplanting did not have a significant effect on Saxifrage growth rates in any interval. Overwinter growth rates were significantly different for control and transplant individuals for Artemisia ($t_{77.577}$=3.293, p=0.001) and Saxifrage (t_{39}=-2.476, p=0.018), although for Saxifrage the difference was related to all of the transplant individuals dying over the winter (Figure A3.2). There was no significant difference in the over winter growth of Chamerion ($t_{59.195}$=-0.672, p=0.505), Carex ($t_{70.885}$=1.712, p=0.091), or Poa ($t_{74.752}$=-1.428, p=0.158).

The nitrogen addition did not significantly affect Artemisia ($t_{117.98}$=0.841, p=0.402), Saxifrage ($t_{111.20}$=-0.019, p=0.984), Carex ($t_{81.225}$=-1.415, p=0.161), Poa ($t_{105.16}$=0.566, p=0.572), or Chamerion ($t_{229.51}$=-0.811, p=0.418) longest leaf growth rates. Inorganic nitrogen fertilizer had no effect on growth rate over the course of the growing season for any of the focal plant species (Figure 3.1). This lack of an effect on growth rate was consistent over the winter as well (Figure A3.2), as none of the focal species had a change in over-winter growth rate due to the nitrogen addition.

Survival

Chamerion had comparable survival for control and transplant individuals in the first interval (control=90%, transplant=100%), but that declined in the second interval (control=85%, transplant=75%), and declined further in the third interval (control=75%, transplant=42%). Survival dropped drastically over the season especially for transplant individuals (Figure A3.3). In the first and second intervals there was not a significant difference in survival for control or transplant (χ^2=1.792, p=0.181), however, in the third interval there was a significant difference between the groups (χ^2=7.428, p=0.006). Chamerion had a significant difference in the over-winter survival of control and transplanted individuals as well (χ^2=6.903, p=0.008). Control plants had a 37.5% survival while transplants had a 10% survival rating (Figure 3.2).

Artemisia had at least 90% survival over the growing season for all control, transplant, and within site transplanted individuals in each measurement interval (χ^2=1.579, p=0.454; Figure A3.3). Artemisia had an 87.5% survival over winter for control plants, while transplants had a 65% survival and within site transplant had a 75% survival (Figure 3.2). However, the difference in survival between the control and transplants was not significant (χ^2=5.565, p=0.062).

Saxifrage had 100% survival in the first interval for all individuals. Saxifrage control plants had 100% survival over the growing season, while transplants had a 30% survival and within site transplants had a 55% survival (Figure A3.3). The difference between transplants and control individuals were significant for Saxifrage over the growing season (χ^2=42.728, p<0.001). Saxifrage did not have any over winter survival for transplanted individuals, and a 75% survival for control individuals (Figure 3.2), which was a significant difference in survivability between control and transplants (χ^2=64.286, p<0.001).

Carex control plants had 97.5% survival, while transplant and within-site transplants had 60% and 75% survival, respectively, by the end of the season, which was similar to the second interval, but drastically reduced from the near perfect survival in the first interval (Figure A3.3). The difference in survival between the control and transplant individuals over the growing season was significant (χ^2=16.521, p<0.001). Carex control plants had an 85% over-winter survival while the transplants had a 32.5% survival and the within-site transplants had a 40% survival (Figure 3.2). The difference between the groups is significant (χ^2=24.545, p<0.001).

Poa control plants had 97.5% survival, while transplant and within-site transplants had 72.5% and 65% survival, respectively, at the end of the season, which also declined from the first interval to the third (Figure A3.3). The difference between the groups was significant (χ^2=12.281, p=0.002). Control individuals overwinter had a 77.5% survival while the transplants had a 27.5% survival and the within site transplants had an even lower 0.05% survival (Figure

3.2). The difference between control and transplants over winter survival was significant for Poa (χ^2=35.129, p<0.001).

Nitrogen addition did not have a significant effect on the survival of any focal species both within the growing season (lowest value: χ^2=2.368, p=0.124; Figure A3.4), and over winter (lowest value: χ^2=1.104, p=0.293; Figure 3.3).

Biomass and tissue nitrogen concentrations

Chamerion, both control and transplant individuals, was the only focal species to allocate biomass evenly between aboveground and belowground tissues (Chamerion control: $t_{20.497}$=0.575, p=0.572; Chamerion transplant: $t_{3.213}$=-1.982, p=0.136), all others had more belowground biomass than aboveground biomass (Carex: $t_{15.408}$=-3.189, p=0.006; Artemisia: $t_{26.532}$=-3.960, p<0.001; Poa: $t_{12.328}$=-3.059, p=0.010). Chamerion was also the only focal species to allocate more carbon to aboveground tissues than belowground tissues (Chamerion control: $t_{19.527}$=5.015, p<0.001; Chamerion transplant: $t_{3.036}$=3.368, p=0.043), the other focal species all had more carbon in belowground tissues (Carex: $t_{23.205}$=-3.393, p=0.002; Artemisia: $t_{48.618}$=-2.630, p=0.011; Poa: $t_{19.995}$=-2.756, p=0.012). All focal species were consistent in allocating more nitrogen to aboveground tissues (Chamerion control: $t_{20.804}$=6.658, p<0.001; Chamerion transplant: $t_{5.593}$=8.742, p<0.001; Carex: $t_{18.815}$=4.358, p<0.001; Artemisia: $t_{49.813}$=5.766, p<0.001; Poa: $t_{17.105}$=7.270, p<0.001).

Since all the Saxifrage individuals died over winter there were no remaining individuals to measure biomass and carbon and nitrogen concentrations. However, Saxifrage species generally have carbon content similar to other alpine forbs and may allocate more carbon to reproductive structures than vegetative structures (Grulke and Bliss 1985). Given that *L* *pyrolifolia* is a rosette saxifrage, it likely has similar properties to those found in *Saxifraga tenuis* and *S. nivalis* by Grulke and Bliss (1985). This indicates that *L. pyrolifolia* likely has similar biomass, carbon, and nitrogen allocations to the other later-successional species.

Nitrogen addition did not affect the nitrogen concentration of above- or belowground tissues for any of the focal species, except for Carex where nitrogen added individuals had less nitrogen in aboveground tissues than control individuals ($t_{9.821}$=-2.808, p=0.019). Similarly, nitrogen addition did not affect the carbon concentration of above- or belowground tissues for any of the focal species, except for Poa where nitrogen added individuals had higher carbon concentrations in belowground tissues ($t_{8.575}$=2.396, p=0.041). There was no difference in the above- or belowground biomass of any focal species between nitrogen added and control individuals.

Soil properties

The soil physico-chemical properties that showed significant difference between the transplant and alpine sites in the 2016 growing season were pH ($t_{3.857}$=6.308, p=0.003), % sand ($t_{4.698}$=-3.888, p=0.013), and % silt ($t_{5.489}$=2.975, p=0.027). C:N ratio was not significant ($t_{3.017}$=3.060, p=0.055), but had such a large effect size difference between the transplant and alpine sites as to be beneficial to acknowledge (Table 3.1). The difference in pH ($t_{3.036}$=4.294, p=0.023) and C:N ratio ($t_{3.001}$=2.907, p=0.062) was also observed in the 2017 growing season. $\delta^{13}C$ was also found to be significantly different between the transplant and alpine sites ($t_{3.610}$=50.222, p<0.001; Table 3.2).

Soil surface and belowground (5 cm depth) temperature was also significantly different between sites during the growing season after controlling for date and time of measurement (surface: χ^2=4499.8, df=2, p<0.001; belowground: χ^2=3477.4, df=2, p<0.001). Soil surface and belowground temperatures were higher on average in the transplant and Saxifrage sites compared to the alpine site. The highest average temperatures were found at the Saxifrage site located farther downslope of the RDG and transplant sites, this is likely due to the low vegetation and farther distance from the glacier terminus. The thermal buffering capacity of the soil, measured as the difference between the surface and belowground temperatures, was also different between sites (χ^2=181.02, df=2, p<0.001). Alpine site soils had a higher thermal buffer than transplant and Saxifrage sites (Figure A3.5) which would help protect the plant roots from extreme temperature changes during the year.

Bacteria:fungi (bac:fun) ratio was not significantly different between sites ($F_{2,18}$=2.292, p=0.130) whereas both bacteria and fungi PLFA's on their own were significantly different between all three sites (bacteria: $F_{2,18}$=86.932, p<0.001; fungi: $F_{2,39}$=77.381, p<0.001). The alpine sites had approximately two orders of magnitude higher PLFA for both fungi and bacteria than did the transplant or RDG sites; although the bac:fun ratios were similar (Table 3.3). The transplant and RDG sites had a higher amount of gram negative bacterial PLFA's compared to gram positive (transplant: t_{14}=4.758, p<0.001; RDG: t_{10}=2.816, p=0.018), however, in the alpine sites this relationship reverses where there are significantly more gram-positive bacteria PLFA's than gram negative (t_{12}=-2.631, p=0.022). Despite their similarities there was a difference in the PLFA communities of the RDG and transplant sites according to an NMDS ordination (Figure A3.6).

Discussion

Growth and survival

The negative transplantation effect observed for most of the focal species in the first growth interval was recovered by the end of the growing season indicating that transplantation did not negatively impact the growth of the focal species long term. Artemisia had a negative effect of transplantation on between season growth indicating that transplanted species did not grow as well as control species. Therefore, the recently-deglaciated terrain (likely through poor nitrogen availability, low temperatures, and limited cover) was unable to support the same level of growth as the later successional areas for *A. norvegica*. Neither of the graminoid focal species had significantly lower between season growth rate for transplants than control individuals. The individuals of these species that survived over-winter were able to grow as well as control individuals from the later successional areas, indicating that the recently-deglaciated terrain was able to support the same level of plant growth for these species, at least over the course of one year. The equivalent growth of surviving graminoids in recently-deglaciated terrain suggests that individuals that can pass the filter of abiotic conditions imposed by the recently-deglaciated terrain are physiologically suited to take advantage of the conditions. This may suggest a specific threshold some individuals exceed that allows them grow just as well in these harsh conditions rather than a gradual effect.

Transplants had a lower probability of survival both within and between seasons for all focal species. The lower probability of survival is due to the differences between the recently-deglaciated terrain and the more developed sites. The drastic change in soil conditions between the sites is likely the cause of transplant mortality in these areas, however, three of the four later-successional species were able to survive over winter in the recently-deglaciated terrain. Though the survival was low for the graminoids, it was fairly high for *A. norvegica* showing a capacity for resilience to changing environmental conditions or tolerance for low nutrient, high pH soil conditions. Perfors et al. (2003) state that sagebrush (*Artemisia tridentata*) growth rate will increase with climate change due to earlier snowmelt dates, allowing the species to potentially shift its range upslope and northward. The enhanced growth of sagebrush is partially attributed to their somewhat woody root system which can create and store organic carbon reserves (Perfors et al. 2003) which is similar to that seen in *A. norvegica* and likely why they survived so well. Regardless of the method employed, the focal species show potential for alpine plants to survive in recently-deglaciated terrain allowing it to act as a temporary haven as treeline advances upslope altering current alpine conditions. The subset of individual plants that are able to survive in these conditions may possess certain adaptations, such as the woody roots of *A. norvegica* which can store carbohydrate reserves (Sturges and Trlica 1978, Wang et al. 2017), or high plasticity. The ability to survive in recently-deglaciated terrain may act as a selection factor for the species and may be vital if treeline continues to displace upslope alpine plants from their current distributions.

Nitrogen addition

The only effects found from the nitrogen addition were decreased aboveground nitrogen concentration in Carex and increased belowground carbon concentration in Poa. Increase in belowground carbon concentration as a result of nitrogen addition has been found previously in grassland ecosystems, which explains why this effect was found in a grass species (Yue et al. 2016). However, reduction of nitrogen concentration due to added nitrogen has not been previously found and mechanistically is unsupported (Bracken et al. 2015). The nitrogen addition had no effect on the focal species growth or survival unlike predicted based on previous studies' results that fertilization positively affected primary production in alpine tundra sites (Bowman et al. 1995, Gough et al. 2000).

The lack of response may have occurred for a few reasons. First, alpine plants can uptake nitrogen for later use which would not be apparent from growth rate measurements (Bowman et al. 1993). This can be ruled out as the tissue nitrogen concentrations were not affected by the nitrogen addition, except in Carex where nitrogen added individuals had a lower aboveground nitrogen concentration. Second, although graminoids have been found to have the greatest response to N additions (Bowman et al. 1993, 1995), some graminoid species respond by decreasing root biomass rather than increasing shoot biomass, effectively reducing their root:shoot ratio without affecting aboveground growth (Bardgett et al. 1999), which would be missed in growth measures. However, both graminoid species had higher root biomass than shoot biomass when collected at the end of the season and the biomass did not change between nitrogen added and control individuals indicating that the nitrogen addition did not affect biomass. Third, nitrogen can experience rapid transformations and turnover in soil and may have been lost before it could be used by either plants or microbes. Fourth, the microbial community may have taken up and immobilized the nitrogen keeping it locked from use by the plant species. Fifth, communities co-limited in nitrogen and phosphorous may respond more to phosphorous additions than nitrogen (Bowman 1994); the soil may be more limited by phosphorous or other nutrients than by nitrogen. And finally, plants in areas of poor nutrient supply may not act favourably to nutrient addition due to their life history and adaptations to nutrient deficient conditions (Chapin 1980, 1991).

Read et al. (2018) found that nitrogen addition did not affect plant community composition when added at an intermediate level for four years. However, it did have a small effect on leaf area index indicating that the plants did increase growth in response to nitrogen. A reason for the increase in growth could be due to the additions occurring over four years (Read et al. 2018), as responses to nitrogen addition get stronger over time (Suding et al. 2008, Farrer et al. 2016).

Bowman and Bilbrough (2001) found that nitrogen added in a pulse, compared to a more even treatment, did not alter the reaction of graminoids to added nitrogen; however, grasses were able to take advantage of higher concentrations of nitrogen while sedges did not change between the low and high nitrogen treatments. The nitrogen addition used in this study was a pulse treatment, and it was added at only two times; once at the beginning and once at the end of the growing season, which may have an effect on the ability of alpine plants to react to the addition. A more constant addition of nitrogen over time may have had a more substantially beneficial effect on the growth and survival of the focal species.

Productivity responses to nitrogen enrichment are primarily due to resource limitation (Farrer et al. 2016). Under this hypothesis the non-significant response we found in our study species after nitrogen addition would have been due to the soils already containing sufficient nitrogen for the plants to thrive. However, based on the soil properties measured, the recently-deglaciated terrain is depleted in nitrogen, both available NH_4^+ and NO_3^- as well as total nitrogen and wide C:N ratio. It seems more likely for our study that the nitrogen added was either stored by the focal species, taken up by the microbial community, released to the atmosphere through gaseous losses, or leached from the soil.

Soil microbial community composition is impacted by nitrogen fertilization (Corkidi et al. 2002, Treseder 2004, Johnson et al. 2008). Mycorrhizal fungi abundance generally decreases while copiotrophic bacteria abundance increases which will have an associated effect on plant productivity and community composition (Leff et al. 2015). Mycorrhizal fungi's role is to increase access to nutrients for plant species in exchange for carbon, in soil with an abundance of resources this relationship becomes more detrimental to the plant species and much less common as a result. The changes in microbial communities due to nitrogen fertilization may drive the response of plants to the added nitrogen, however, it is not likely the explanation for the plant response in this study for a number of reasons. First, the microbial community needs time to adapt to the changing nutrient conditions of the environment, and second, microbial communities are more strongly impacted by a change in plant composition than soil nutrient status. The changes in microbial composition are driven by the changes in plant community composition as a response to the added nitrogen (Bardgett et al. 1999). However, this relationship does explain the pattern of altered microbial communities between transplant and RDG sites identified from PLFA (figure A3.8). The transplanted specimens still contained some of the soil on their roots from their original locations which changed the microbial community in the areas they were transplanted into. Although likely only a subset of the microbial community brought over was able to survive in the recently-deglaciated terrain, the PLFA profile in alpine soils is comparable to the RDG sites.

Conclusions and future research

Three of the four focal species were able to survive for one year in recently-deglaciated terrain suggesting a potential for these areas to acts as refugia for alpine vegetation threatened by upslope treeline migration associated with climate change effects. The lack of plant response to the nitrogen addition indicates that the focal species that were able to survive in the recently-deglaciated terrain could do so under the current nutrient dynamics of those sites and do not rely on the increased nitrogen pool that comes from continual and escalating atmospheric nitrogen deposition associated with anthropogenic activities. Whether these plants will move into recently deglaciated areas is dependent on whether their seeds can germinate in these conditions, and whether other species will outcompete them until there are more established nutrient and microbial pools. Seed germination and plant establishment may still prevent later-successional alpine plants taking advantage of the newly exposed terrain. In particular, sedge species may be less able to germinate in arctic-alpine conditions (Bliss 1958). Both seed and establishment limitation have previously been found within glacier forelands for all seral stages / age classes present (Jones and del Moral 2009). Future research should focus on germination of seedlings of later-successional alpine plant species in recently-deglaciated terrain and competition between later-successional and early-successional species. Studies on seed rain dynamics and potential for these plant species to migrate upslope into the recently-deglaciated terrain as well as their establishment via germination are necessary to better understand the coming changes in plant composition along the gradient from recently-deglaciated to alpine areas. Research into the strength of recently-deglaciated terrain as an evolutionary selection factor acting on later-successional species that can survive in these areas would also be of interest for future studies.

Tables

*Table 3.1. Average and standard error of all soil properties measured in 2016. Significance denoted as: * - $p<0.05$, • - $p<0.10$.*

Site	pH	EC	NH_4	NO_3	TN	TC	C:N	$\delta^{15}N$	% clay	% silt	% sand
		dS m^{-1}	mg kg^{-1}	mg kg^{-1}	mg mg^{-1} %	mg mg^{-1} %		‰			
	*						•			*	*
RDG	7.99±0.15	0.080±0.006	0.44±0.01	0.19±0.08	0.00±0.002	0.48±0.06	120.59±33.38	NA	14.8±0.3	53.0±3.9	32.0±3.8
Alpine	5.31±0.40	0.045±0.018	0.56±0.10	0.12±0.04	0.32±0.11	6.41±2.53	19.33±1.77	4.65±0.65	12.3±1.4	38.5±2.9	48.8±2.1

*Table 3.2. Average and standard error of all soil properties measured in 2017. Significance denoted as: * - $p<0.05$, • - $p<0.10$.*

Site	pH	EC	NH_4	NO_3	TN	TC	C:N	$\delta^{15}N$	$\delta^{13}C$
		dS m^{-1}	mg kg^{-1}	mg kg^{-1}	mg mg^{-1} %	mg mg^{-1} %		‰	‰
	*						•		*
RDG	9.17±0.06	0.121±0.072	1.02±0.11	0.71±0.10	0.002±0.00	0.52±0.04	354.74±114.91	NA	-5.83±0.35
Alpine	5.61±0.83	0.046±0.011	3.35±1.33	5.61±2.57	0.36±0.15	7.00±3.15	20.64±1.71	5.76±0.56	-24.53±0.11

Table 3.3. Mean and standard error for bacterial and fungal phospholipid fatty acids (PLFA) and the ratio of bacterial to fungal PLFA's (Bac:Fun) for RDG-transplant, RDG, and alpine sites. RDG-transplant refers to the RDG sites later-successional plant species were transplanted into, whereas glacier edge refers to unaltered recently-deglaciated soil and Heath-grassland refers to unaltered alpine soil.

Site	Gram -	Gram +	Total Bacteria	Fungi	Bac:Fun
	nmol g^{-1}	nmol g^{-1}	nmol g^{-1}	nmol g^{-1}	
RDG-transplant	14.46±1.99	4.13±0.88	18.59±2.74	5.59±0.88	3.15±0.16
Glacier edge	6.63±1.87	1.13±0.57	7.76±2.42	2.62±0.60	2.81±0.31
Heath-grassland	435.16±51.39	696.35±84.96	1131.51±135.89	329.31±36.91	3.43±0.11

Figures

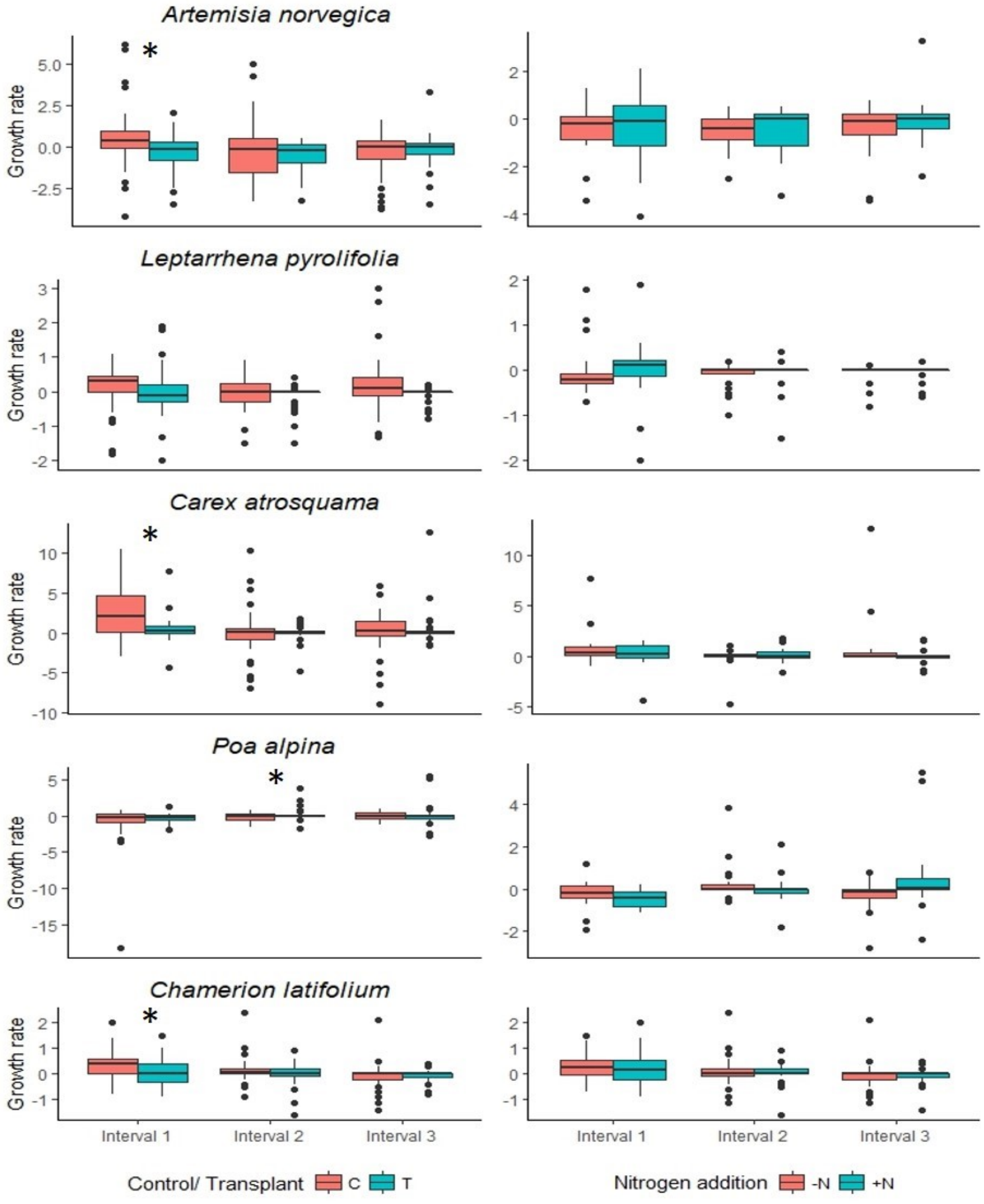

*Figure 3.1. Growth rate of five focal species in three two-week intervals over the 2016 growing season. Control and transplant individuals, as well as control and nitrogen added individuals, were compared for each of the five focal species. Transplantation affected the first growth interval for Artemisia, Carex, and Chamerion, as well as the second interval for Poa. Nitrogen addition did not affect the growth rate of the focal species. * indicates significant difference (p<0.05) in interval.*

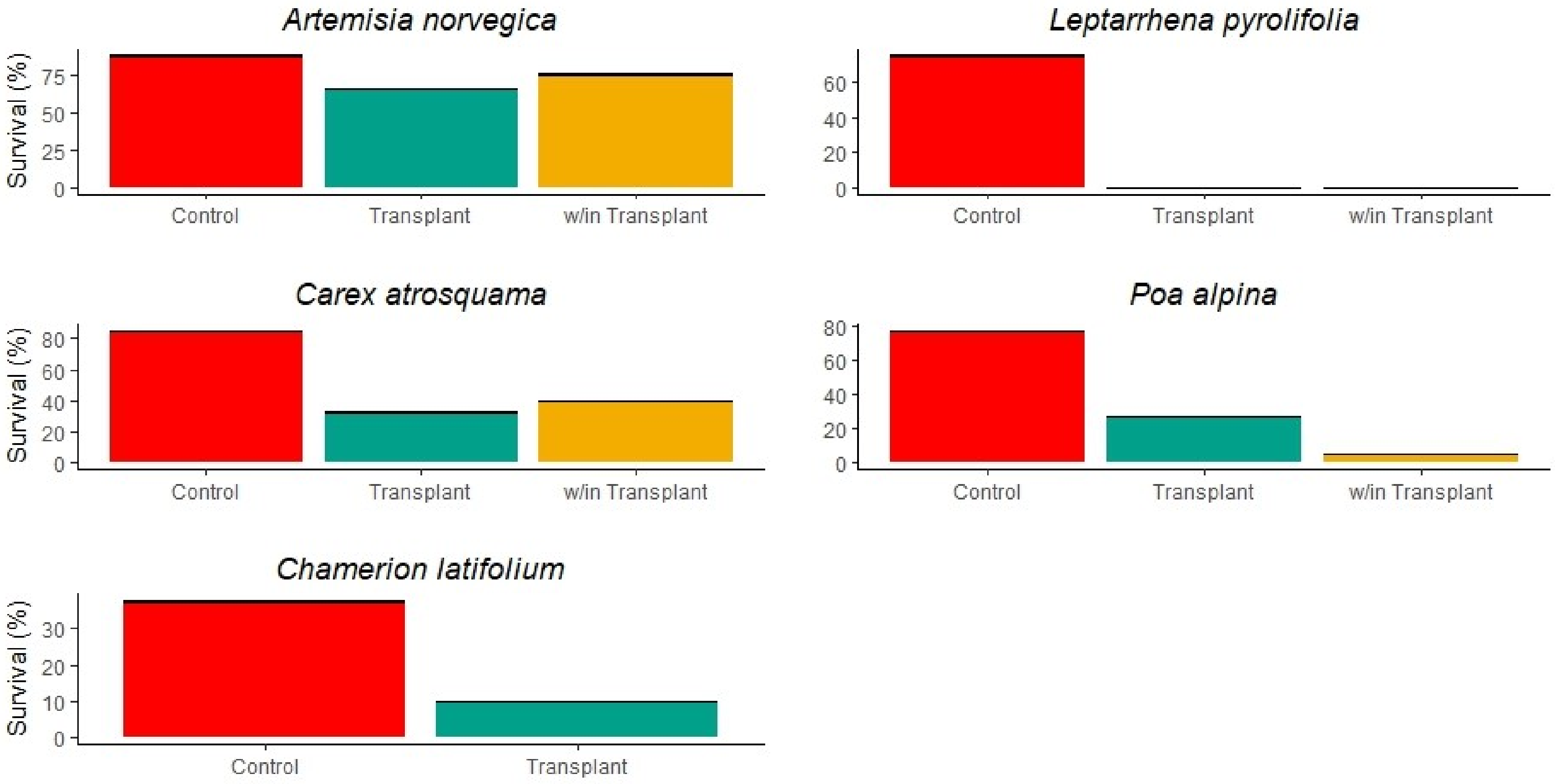

Figure 3.2. Percentage of over winter survival for five focal plant species for control, transplant, and within site transplant of individuals. Transplanted individuals had lower over winter survival success than control plants for each of the focal species. Within site transplants fared better than RDG site transplants for Artemisia norvegica and Carex atrosquama, however Poa alpina within site transplanted individuals had higher mortality than RDG site transplants. Three of the later-successional plants were able to survive over winter in the recently-deglaciated sites; only Leptarrhena pyrolifolia was completely eradicated. There is potential for these plant species to survive in recently-deglaciated terrain as treelines advance and glaciers recede.

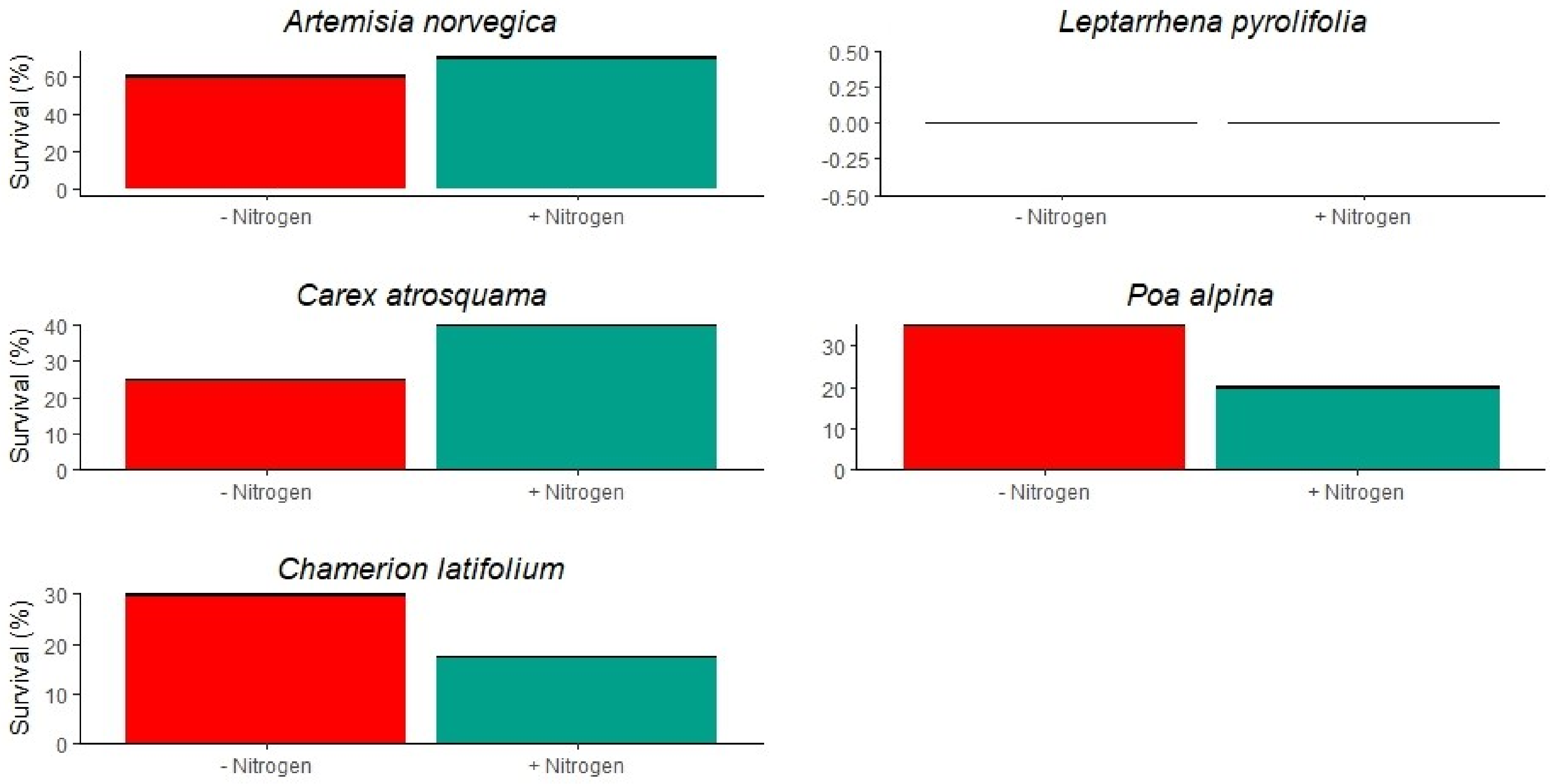

Figure 3.3. Percentage of over winter survival for five focal plant species for control and nitrogen added individuals. Nitrogen addition did not significantly affect the proportion of surviving individuals for any focal species.

Appendix

Table A3.1. Table depicting the focal plant species included in the study and which sites they were found and under what experimental treatments they were subjected to. Chamerion latifolium was used as a control species by representing an early successional species already found in the four RDG sites. Half of the individuals were transplanted within site, and half of those transplants had nitrogen added as ammonium nitrate. The other four focal species were all found at separate alpine sites with forty individuals then transplanted across the four RDG sites, with half of those individuals supplied with additional nitrogen as ammonium nitrate. Forty individuals of each late species were also measured within their original growing sites, and half of those were also transplanted within their original sites.

Focal species	RDG Sites				Alpine Sites	
	Control		Transplant		Control	Transplant
	+N	-N	+N	-N	/	/
Chamerion latifolium	20	20	20	20	/	/
Leptarrhena pyrolifolia	/	/	20	20	20	20
Poa alpina	/	/	20	20	20	20
Carex atrosquama	/	/	20	20	20	20
Artemisia norvegica	/	/	20	20	20	20

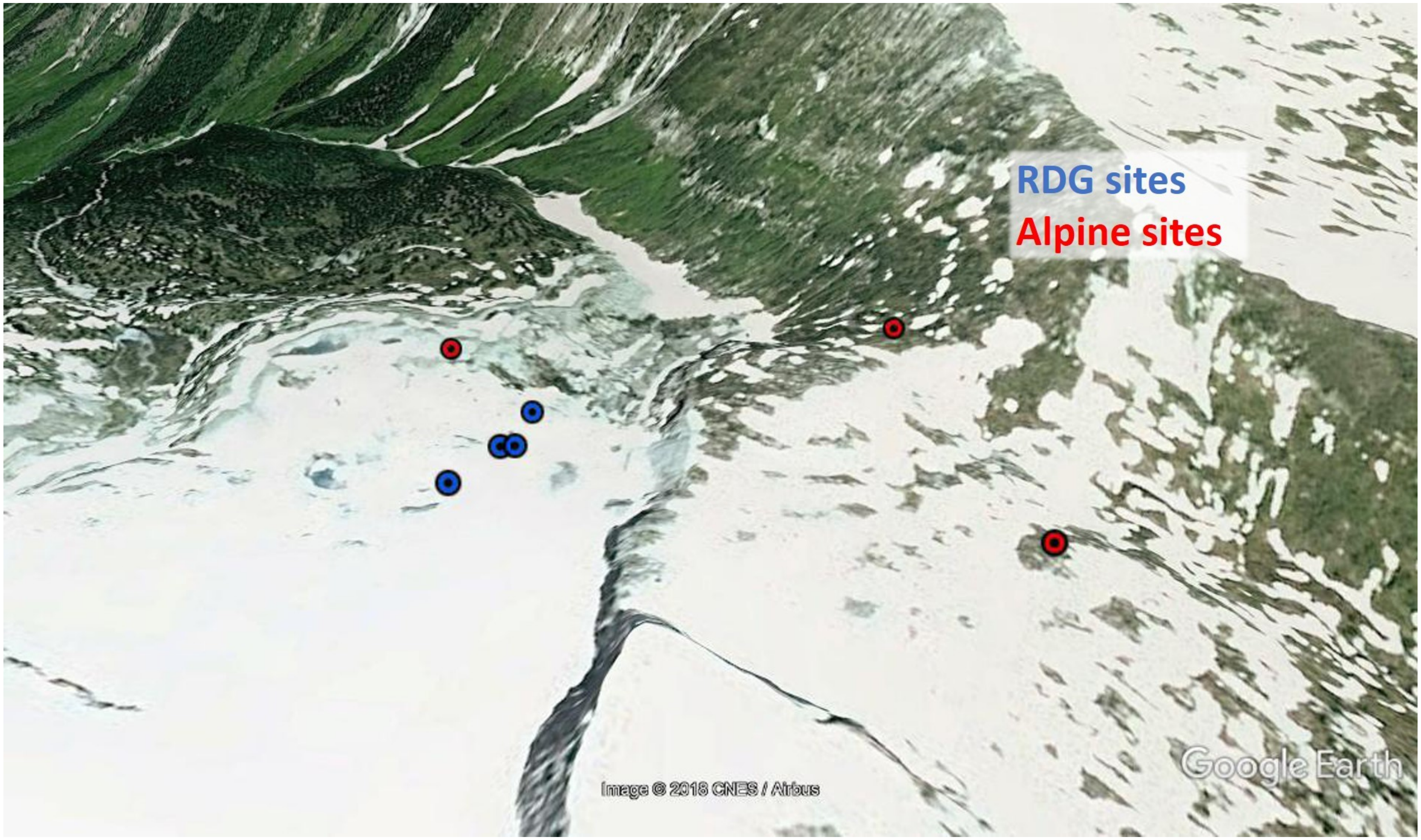

Figure A3.1. Map of Bachelor Pass with the four RDG sites (blue) and four alpine sites (red) depicted. Individuals of the four later successional focal species were transplanted from the alpine sites into the RDG sites at the foot of the easy glacier. Image was acquired from Google Earth with the sites labelled in the map directly.

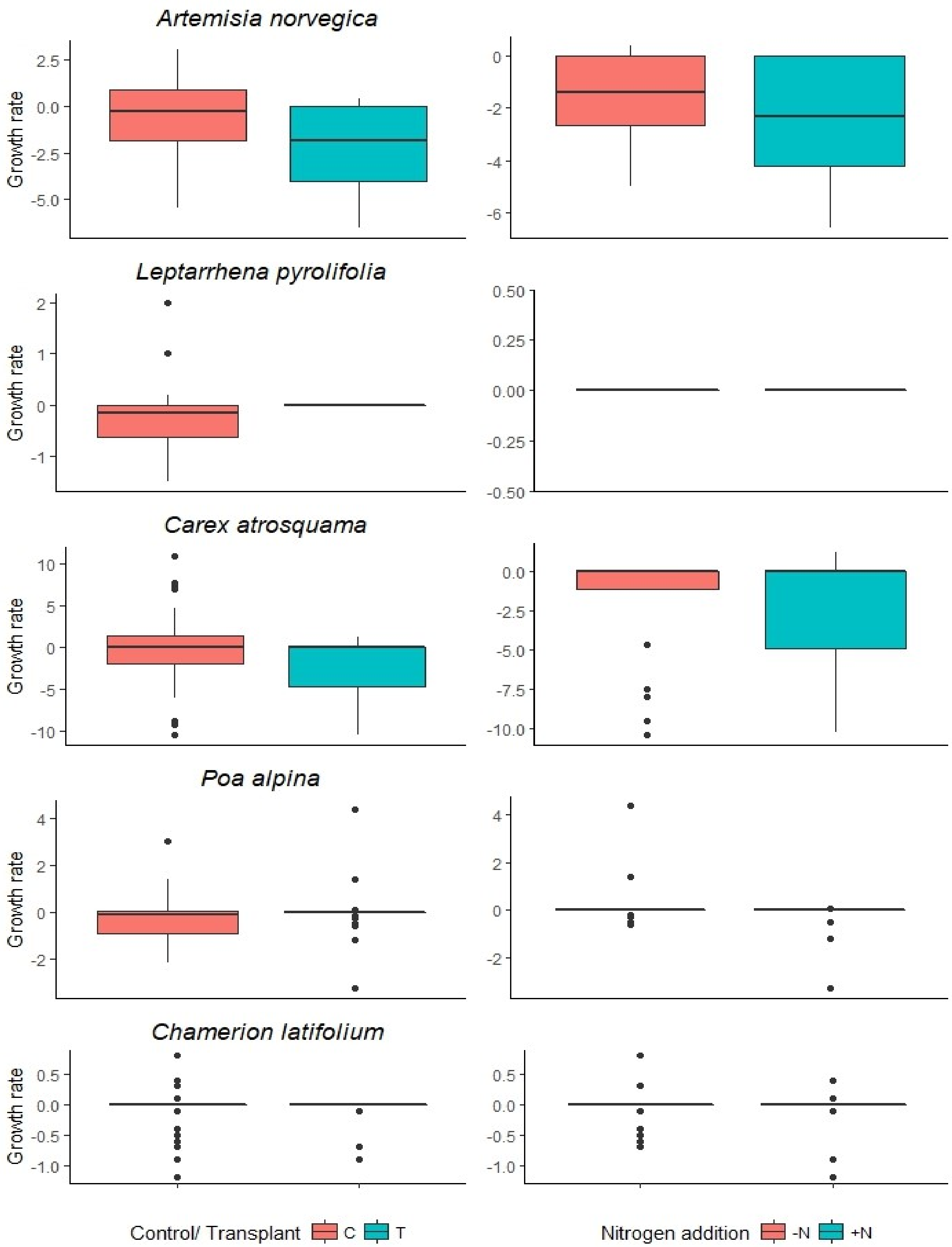

Figure A3.2. Growth rate of focal species over-winter between the 2016 and 2017 growing seasons. Control and transplant individuals, as well as control and nitrogen added individuals, were compared for each of the five focal species. Nitrogen addition was found to have no effect on the growth rates of any species, and transplantation had an effect on Artemisia norvegica and Leptarrhena pyrolifolia, though the latter is likely due to all of the transplants dying over the winter.

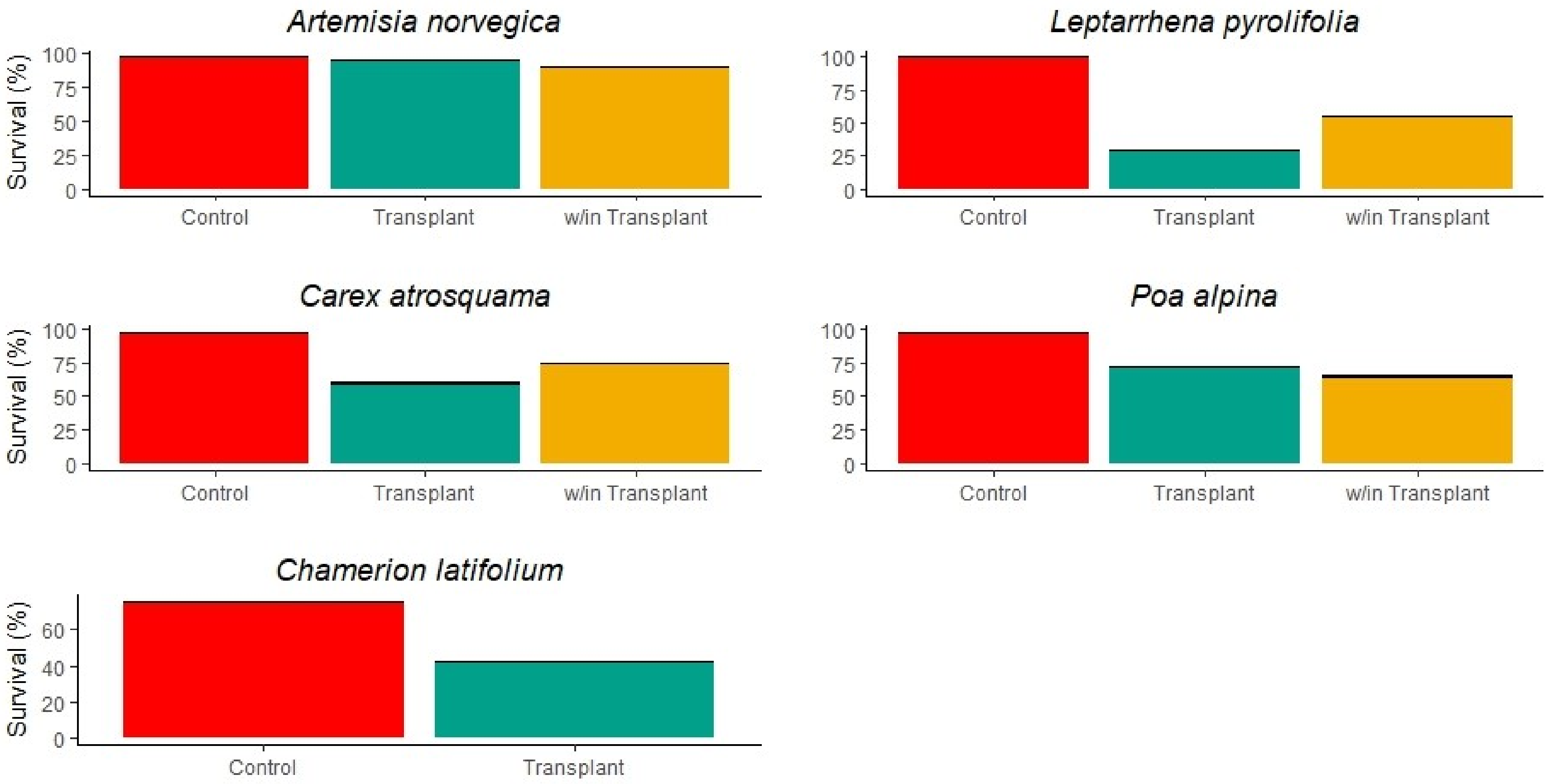

Figure A3.3. Percentage of within season survival for five focal plant species for control, transplant, and within site transplant of individuals. Artemisia norvegica had the highest proportion of surviving transplanted individuals. All focal species had high survival for control individuals, with Chamerion latifolium having the lowest survival for control plants. Chamerion latifolium and Leptarrhena pyrolifolia were the only species to have mortality outweigh survival for transplants.

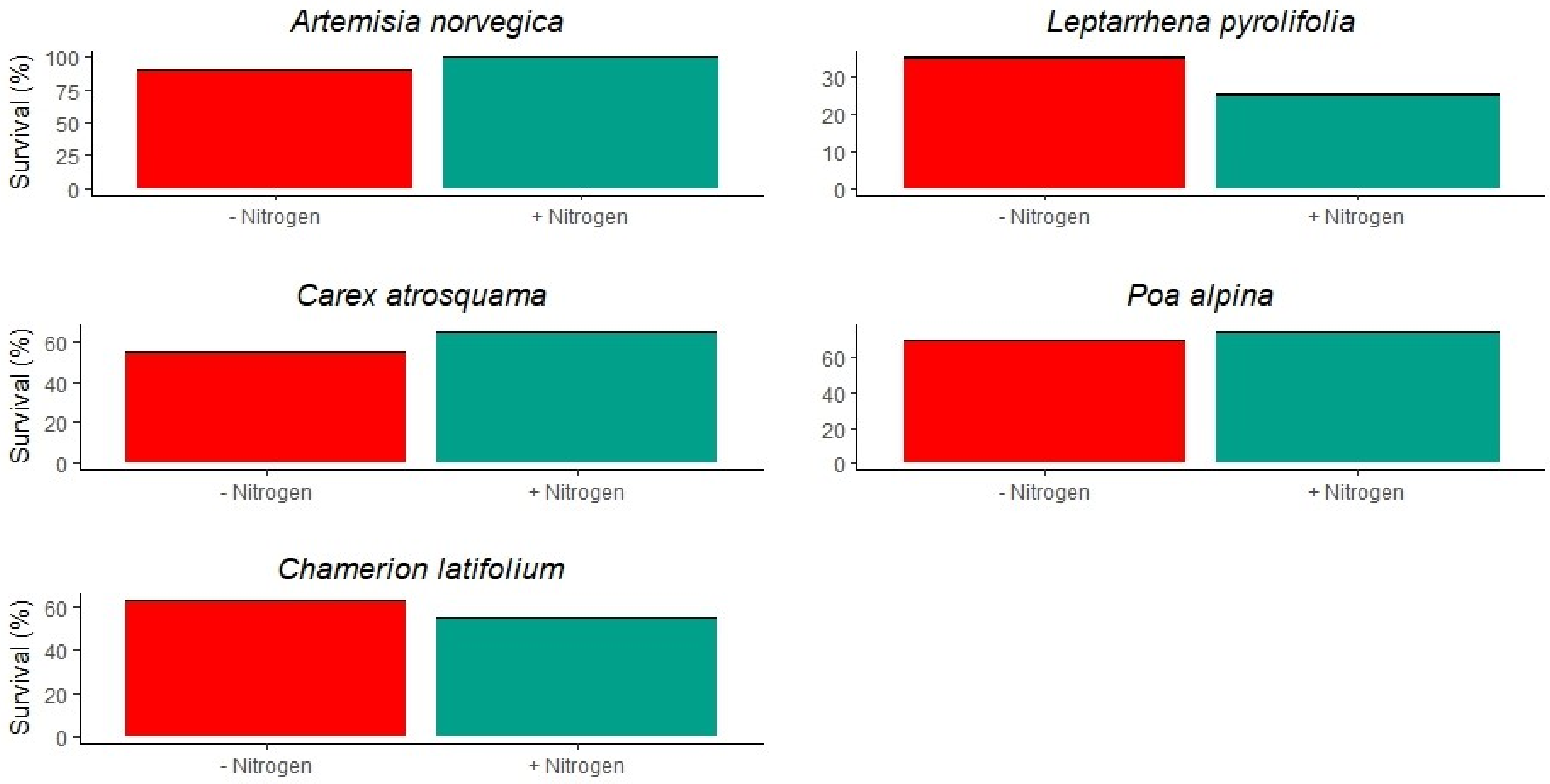

Figure A3.4. Percentage of within season survival for five focal plant species for control and nitrogen added individuals. Nitrogen addition did not significantly affect the proportion of surviving individuals for any focal species.

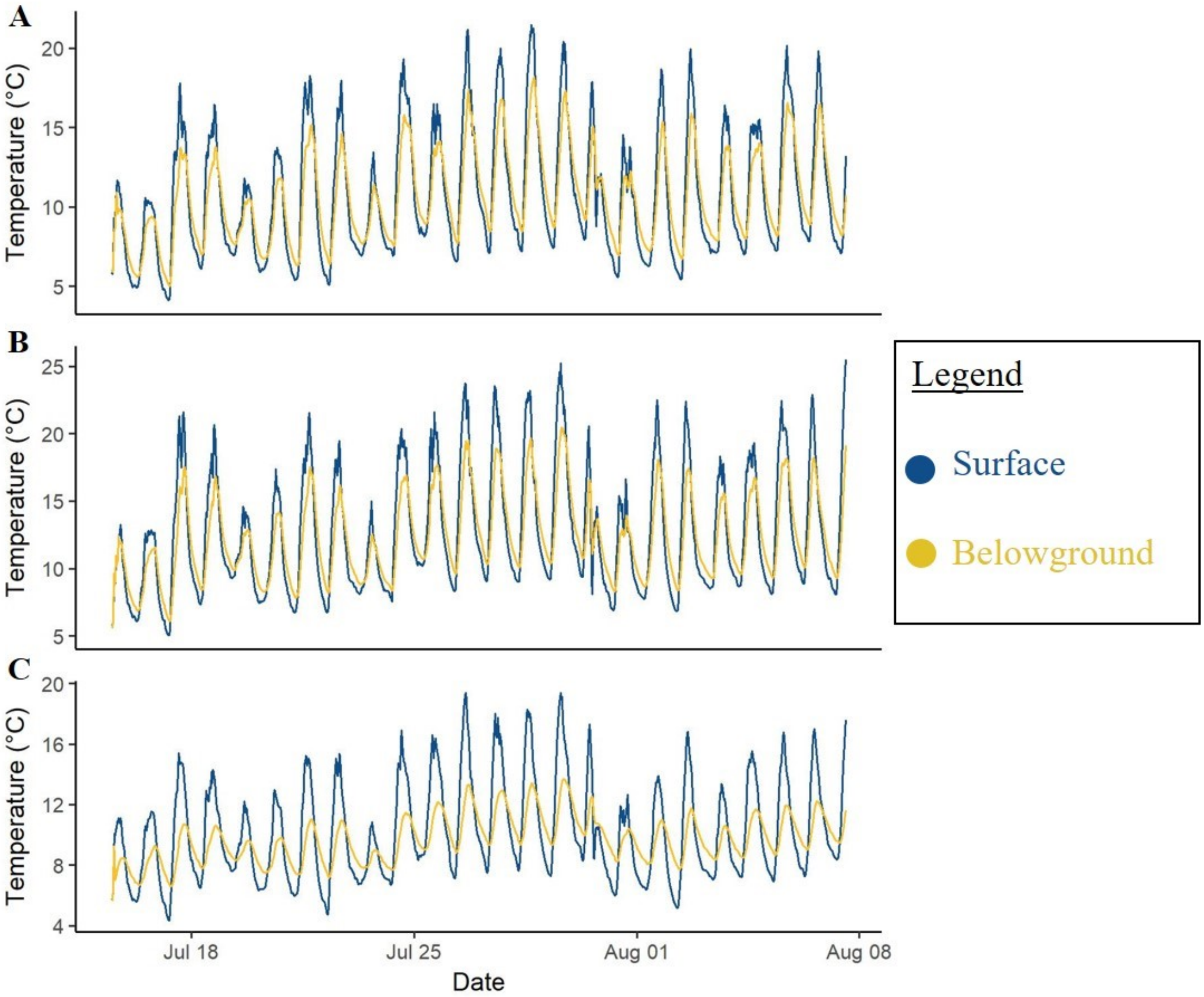

Figure A3.5. Surface and belowground (-5cm) soil temperatures from July 15 – August 7, 2016. A) RDG site 1 located near the terminus of the Easy Glacier. B) Saxifrage site located farther down the valley from the glacier still within relatively recently-deglaciated soil. C) Alpine site located at the edge of Bachelor Pass over-looking the transects downslope to the treeline. The alpine site with its more developed soil was more well insulated than the earlier sites. Though the belowground soil temperature still varied on a diurnal pattern it was much less pronounced than the diurnal variation of the soil surface temperature indicating that the rooting zone, particularly in the later successional areas, is a more constant thermal environment.

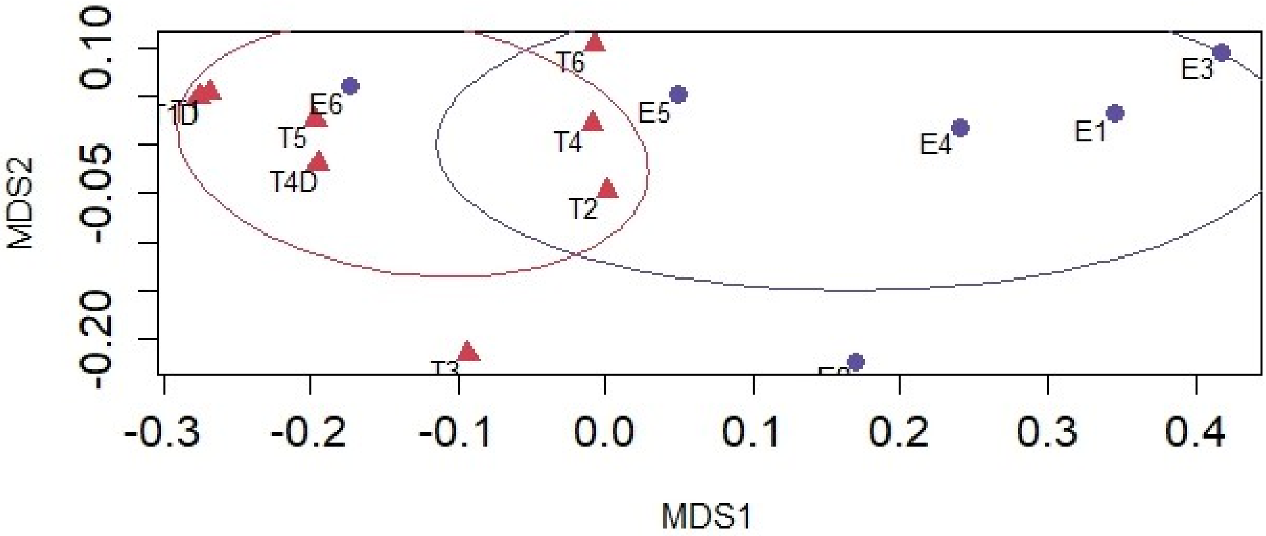

Figure A3.6. NMDS ordination of RDG and alpine site PLFA's determined by GC-mass spectroscopy. There is a difference in the clustering of sites based on the PLFA's present. Transplant sites differ from RDG sites in the PLFA's that are found there despite both site types located on recently-deglaciated terrain. The difference between the sites is likely due to microbes brought over with the transplant species from the later-successional areas, nitrogen added at the Transplant sites, and plant-soil feedbacks occurring over the winter at the transplant sites. Though there is a consistent difference in PLFA assemblage, site E6 was found to have a similar community to the Transplant sites which suggests that the transplant microbial communities are still within the range of variation that can be found in the recently-deglaciated terrain naturally. Ellipses correspond to the grouping of the data, where red: Transplant sites and blue: RDG sites. Points labelled E correspond to RDG sites, while sites labelled T or T#D refer to the transplant sites.

Chapter 4

Soil CH_4 uptake in alpine temperate regions contributes to global methane consumption: spatial variation, driving mechanisms, and potential implications

Introduction

Rapid global climate change is occurring because increasing anthropogenic greenhouse gas concentrations in the atmosphere trap heat and increase climate forcing (Crowley 2000, Tian et al. 2016). The primary biogenic greenhouse gases are CO_2, CH_4 and N_2O, and all three have significant contributions from soils (Oertel et al. 2016). In most parts of the world, mountains are warming at an accelerated rate compared to lowland areas; a process called elevation dependent warming (Pepin et al. 2015). Little is known about the CH_4 and CO_2 fluxes in temperate alpine regions, including western North America. The balance between emission and uptake from soil for both of these greenhouse gases may determine the feedback responses to further climate change in mountains. Improving these estimates will also contribute to development of better global and regionally sensitive climate models.

Soil fluxes are the net uptake or release of a gas between the soil and the atmosphere. These gas exchanges are composed of and in part driven by both the microbial and physical aspects of the soil (Smith et al. 2003). Microbes are responsible for the production or consumption of different gaseous products which are then able to diffuse into or out of the soil depending (generally) on concentration gradients. However, the rate of diffusion is generally controlled by the pore size, aggregate size, and amount of water filled pore space in the soil

(Smith et al. 2003, Venterea and Baker 2008). In this way soil fluxes result from a combination of the microbial processing and physical movement capability of gas in soil.

Alpine regions comprise approximately 3% of total mountain area which in turn compose 25% of the global land area (Hu and Bliss 2018). Alpine regions are very heterogenous in terms of topography, vegetation, and edaphic properties and so are ideal locations for the investigation of the drivers of greenhouse gas fluxes from soils. Differences in the type of soil, terrain elevation, and time since deglaciation across natural spatial gradients allow for a more comprehensive examination of the mechanisms driving CH_4 and CO_2 fluxes. Recently-deglaciated Regosolic soils are being formed and becoming more available due to progressive glacial recession leading to newly exposed terrain (Chersich et al. 2015). This is caused by an accelerated warming rate in these temperate mountain landscapes with nearly double the warming rate of the global average (Rangwala and Miller 2012, Spasojevic et al. 2013, Pepin et al. 2015). These recently-deglaciated areas may represent the initial baseline in terms of CH_4 and CO_2 fluxes as they are comparatively barren in terms of vegetation and microbial communities. Alpine tundra has comparatively more developed soils, often Brunisols, and are primarily composed of graminoids and heath vegetation. The vegetation in these areas is potentially threatened due to climate change as they are adapted to high elevation, cold habitats (Gottfried et al. 2012, Malanson et al. 2015). Climate change mitigating or enhancing effects of greenhouse gas fluxes may have a large impact on the state of these ecosystems in the future. Along an elevation gradient, the alpine zone slopes through the subalpine towards montane forest which have different soil characteristics due to time since deglaciation and vegetation differences. The montane forest zone is thought to be either expanding upslope with treeline advance or becoming more heavily vegetated with tree infilling (Cannone and Pignatti 2014, Camarero et al. 2017).

These changes in spatial distribution along an elevational gradient may indicate the upcoming changes in the subalpine and alpine zones over time in terms of both vegetation dynamics and CH_4 and CO_2 fluxes.

CH_4 and CO_2 fluxes in temperate alpine ecosystems have been previously studied, however the majority of the work has come from China (Wei et al. 2015, Zhu et al. 2015, Wu et al. 2017, Fu et al. 2018), Europe (Kitzler et al. 2006, Koch et al. 2007, Hofmann et al. 2016a, Chiri et al. 2017, Mutschlechner et al. 2018), and Colorado (Brooks et al. 1997, Knowles et al. 2015, Lin et al. 2017). There were not any studies concerning alpine CH_4 and CO_2 soil fluxes in Canada found at the time of searching, let alone within the Columbia Mountains. The gap in alpine literature on Canadian mountain flux dynamics needs to be highlighted and further research should elucidate patterns and driving processes of alpine soil fluxes in Canadian mountains. In order to make accurate predictions regarding future climate change a large range of temperate alpine sites should be established and included to determine the variation between areas accurately.

Understanding the contribution of alpine fluxes is necessary for more accurately estimating global greenhouse gas balances. The variabilities of CH_4 and CO_2 fluxes along environmental gradients in the Canadian alpine are unknown and required to estimate the contribution of alpine regions. Determining the mechanisms driving the fluxes along the gradient can further help estimating fluxes in understudied alpine regions.

The overall objective of the study was to evaluate the direction, intensity, and variation in alpine soil CH_4 and CO_2 fluxes in the Columbia Mountains, Canada. Specifically, to compare the CH_4 and CO_2 fluxes of recently deglaciated (RDG sites) and more developed (alpine sites) soils, as well as along an elevational gradient split into three bands (high, mid, and low) within the peak of the growing season (summertime). The physico-chemical soil mechanisms driving the CH_4 and CO_2 fluxes in these sites were also investigated. It was initially hypothesized that RDG and alpine sites differ in magnitude of both CH_4 and CO_2 fluxes due to the large differences in vegetation and edaphic variables between the sites. We also hypothesized that the elevational bands differ between each other, but not consistently for CH_4 and CO_2 fluxes as they are driven by different soil properties. Vegetation, soil moisture (as a reverse proxy for aeration), and temperature are putative driving factors of the differences in CH_4 and CO_2 fluxes between sites as they have previously been identified as strong predictors (Borken et al. 2003, Parkin and Kaspar 2003). The ultimate goal of this study is to document the direction and magnitude of CH_4 and CO_2 fluxes in the Columbia Mountains and identify mechanisms driving these fluxes. These quantified methane fluxes will then be compared within available information about methane exchange from other biomes and ecosystems to provide a generalization and broader global context of our results and to gain insights into the potential impact of alpine areas.

Methods

Field and Laboratory Measurements

Field measurements were conducted at the peak of the growing season in August 2017 in Bachelor Pass, British Columbia, Canada (51° 31'21.06"N; 117° 57'31.55" W). Flux measurements were conducted in three elevational bands ("high", "mid", and "low") along two transects on the east side of the pass, as well as in the recently-deglaciated terrain near the terminus of the Easy Glacier (RDG sites) and in the more developed terrain covered with alpine vegetation on the western edge of Bachelor Pass (alpine sites) (Figure A4.2). The alpine sites are located at the top of the pass around 2100 m a.s.l. Likewise, the high band is approximately 2100 m a.s.l, the mid band is around 2050 m a.s.l, and low band is approximately 2000 m a.s.l. RDG sites were composed of recently-deglaciated terrain with limited nutrient levels and vegetation cover and are primarily composed of grey silty soils of the class Regosol. Alpine sites have much higher vegetation cover than RDG, mostly graminoids and heather, as well as more developed soil with higher nutrients, horizonation and more organic matter classified as Brunisols (Table 4.1; Figure A4.1). The transects contain more vegetation types than the alpine sites and are located on more topographically diverse terrain as they slope towards a valley in the east; their soils would also be classified as Brunisols. The high band contained vegetation similar to the alpine sites, while the mid and low bands contained more varied vegetation such as more wildflowers, shrubs, and trees farther downslope.

A dynamic closed-chamber method was used to measure the water vapour, CH_4 and CO_2 fluxes in this study where chambers, composed of two sections: a base and a top both composed of PVC plastic, were installed in the study plots. Each chamber contained a gasket and rubber band for sealing the top to the base, as well as vent tubing to equalize pressure to the atmosphere and reflective tape to reduce the effect of warming from sunlight. Five chambers were installed in each of two sub-plots in the RDG site, for a total of ten RDG site chambers. Ten chambers were also installed in the alpine sites following the same arrangement as the RDG sites. Three chamber bases were installed in each of three elevational bands [high, mid, and low] in the first transect. For the second transect three chamber bases were installed in the low elevation band, four at the intermediate elevation band, and the high elevation chambers in the first transect were measured two times for each day of measurements.

Flux measurements were conducted *in situ* in early August 2017 at near the peak of the growing season during both mornings and afternoon of three consecutive days using a portable laser analyzer (GasScouter G4301, Picarro Inc, Santa Clara, CA, United States) connected to plastic flux chambers for gas recirculation using Teflon one-fourth inch O.D. tubing. The laser analyzer recorded both the CO_2 and CH_4 mixing ratios on dry basis in each chamber location. Chamber bases (8.3 cm height x 16 cm diameter) were installed roughly 5 cm deep in the soil one day prior to measurements and left for the duration of the field sampling protocol. Bases were installed in areas either naturally or artificially devoid of vegetation to determine the contribution of the soil only to CH_4 and CO_2 fluxes. Chamber tops (11.5 cm height x 16 cm diameter) were placed and sealed onto the bases when flux measurements were taken, each measurement interval was five minutes to allow for adequate mixing of gas and an accurate non-steady state flux to be recorded. Chamber tops were then flushed with one minute of ambient air afterward in preparation for the next flux measurement. The laser analyzer records a measurement of gas concentration every 1.25 seconds. The time series of gas concentrations measured in each five-minute measurement interval were plotted to visualize and select the actual period when gaseous flux was calculated. Periods of few seconds of data during the closing and opening the chambers were discarded as data during these periods are associated with disturbances of the gas recirculation flow. Following visual inspection linear regressions were fitted to the data using R package Flux (Jurasinski et al. 2014), in order to estimate the actual flux for both CH_4 and CO_2 based on the derived regression coefficients and the ideal gas law. For the CO_2 measurements 95% of the 177 measured fluxes had an R^2 higher than 85%, although for the CH_4 measurements only 26% of the 177 measured fluxes had an R^2 higher than 85%. However, the methane fluxes that were below an R^2 of 85% were non-significant, whereas each significant methane flux had an R^2 higher than 85%.

Soil temperature, volumetric water content, ground surface temperature, altitude, and ambient pressure were recorded *in situ* at each chamber location (n=36). Soil temperature and volumetric water content were measured with a Steven's HydraProbe, surface temperature was measured with an Apogee MI-230 infrared radiometer, altitude and air pressure was measured using a Testo 511 handheld altimeter (Table 4.1). Composited soil samples (n=36) were also collected from each chamber location and analyzed for the following properties: total nitrogen (TN), total carbon (TC), carbon:nitrogen ratio (C:N), $\delta^{15}N$, NH_4^+, NO_3^-, pH, electrical conductivity (EC), and texture (Table 4.1). pH and EC were measured by mixing 10 g of each soil sample with 20 mL of water for a 2:1 water:soil ratio and determining the pH and EC of the resulting mixture with a calibrated Fisher AR 20 pH/EC meter. Available ammonium and nitrate were determined by a nutrient extraction with 50 mL of KCl mixed with 5 g of air-dried soil per sample and shaken for 30 minutes. The resulting mixtures were then filtered and NH_4^+ & NO_3^- concentration was determined via colorimetric assay. TN, TC, and C:N ratio as well as $\delta^{15}N$ were all measured using a Costech 4010 Elemental Analyzer following the dry combustion method. Soil texture was determined by hydrometer method, with measurements taken at the 40 second and 7 hour intervals to accurately determine % of sand, silt, and clay within the samples. Vegetation cover (%) and dominant species cover at each flux chamber was determined from visual interpretation and scoring of plot photographs for each chamber base location using a standard area size of 50x50 cm. Composited soil samples were also collected using ethanol sterilized tools and whirlpaks to be used in a phospholipid fatty acid (PLFA) analysis to determine the changes in microbial community, between RDG, and alpine sites in the 2017 season. These soil samples for microbial analyses were kept on ice in a cooler for less than 48 hours total before being transferred to a -80°C freezer at the University of Alberta. Following extraction of the PLFA's, samples were analyzed using gas chromatography (GC) to determine the PLFA's present in each sample.

Statistical Analysis

The flux measurements for both CH_4 and CO_2 were subjected to multiple comparisons between site types and times of measurements. T-tests were used to compare between the RDG and alpine sites and between the morning and afternoon measurements. One-way ANOVA was used to compare between days, elevational bands along the transects, and between individual chambers, after performing Kruskall-Wallis tests for normality.

Potential mechanisms driving soil fluxes for both CH_4 and CO_2 were analyzed using regression trees and path analyses. The regression trees were constructed using both the rpart and party packages from R (Hothorn et al. 2006, Therneau et al. 2015, R Core Development Team 2017). Both packages use recursive partitioning to construct the trees, however rpart uses the Gini index to evaluate the splits in the data while party uses permutation tests to select variables. Rpart and the Gini index has been criticized as often overfitting data, whereas party reduces overfitting with a p-value criteria the splits must pass; however, it also forces the data to be evaluated by permutation significance tests that may not be appropriate for all data. Using multiple lines of evidence helps to reduce bias inherent in a single test (Munafò and Davey Smith 2018). The common variables selected by both regression tree tests will be the strongest explanatory variables for the flux data and focused on as the primary drivers. Variables selected by one test but not the other are more tenuous potential drivers that may be more site specific or complex. Regression trees are effective for analyzing data as the recursive nature allows them to identify complex relationships within the data, and can perform better than logistic regression (Karels et al. 2004, Mitchell et al. 2009). Also since regression trees are non-parametric they do not make assumptions about data distribution and are able to work with incomplete datasets (De'ath and Fabricius 2000, Karels et al. 2004, Mitchell et al. 2009). Variables used in construction of the trees were all remaining variables after selection by variance inflation factors (VIF), where the VIF's for all properties were determined, the largest removed, and then all the properties checked again. This selection process continued until all remaining variables were found to have VIF's below 4 which removed all cases of multicollinearity between variables. The remaining variables were used to construct the regression trees. Since the variables were identical for both the CH_4 and CO_2 fluxes, as they were measured concurrently, the properties that remained for analysis were: soil temperature, soil moisture, NO_3^-, % clay, % vegetation cover, and primary vegetation (the species with the highest percentage cover at each site). Path analyses were constructed using the variables that were shown to be important by the regression trees using the lavaan package in R (Rosseel 2012).

Results

Flux Comparisons across Field Locations and Measurement Dates

The CH_4 and CO_2 fluxes did not differ between the four days of measurements (CO_2: $F_{1,175}=2.525$, $p=0.114$; CH_4: $F_{1,175}=0.004$, $p=0.949$; Table A4.1). Likewise, there was no difference between the fluxes at different times of the day (morning or afternoon) for CH_4 ($t_{148.81}=-6.051$, $p=0.546$). There was an effect of time of day on CO_2 measurements ($t_{171.51}=2.692$, $p=0.007$), with significantly stronger CO_2 efflux occurring in the afternoon compared to morning for the RDG sites ($t_{57.087}=-4.556$, $p<0.001$). However, for the alpine sites and high elevational band transect sites the effect was not significant ($t_{52.78}=-1.991$, $p=0.052$; $t_{15.992}=-0.886$, $p=0.389$, respectively).

The significant difference between the RDG site methane fluxes and alpine site methane fluxes ($t_{59}=7.060$, $p<0.001$) was due to RDG sites not having any significant methane fluxes (Figure A4.3). Methane fluxes were also significantly different between the elevational bands along the transects ($F_{2,54}$=43.64, p<0.001; Figure A4.4). A *post hoc* Tukey HSD test showed that the middle elevational band has significantly larger methane uptake than the high and low elevational bands which were not different from each other (p<0.001). The middle elevational band had approximately four times greater methane uptake than the high band; the low band did not register any significant methane fluxes. A significant difference in the CO_2 fluxes was also found between the RDG and alpine sites ($t_{60.753}$=-12.683, p<0.001). The mean CO_2 efflux in the alpine sites was over 1.5 g m^{-2} hr^{-1} greater than in the RDG sites. The CO_2 fluxes also differed along the transects, with the high elevational band having significantly lower CO_2 emissions compared to the mid and low bands ($F_{2,54}$=52.41, p<0.001; Figure A4.4). A *post hoc* Tukey HSD test showed that the high elevation site had nearly three times lower CO_2 emissions than the mid and low elevational bands (p<0.001).

Mechanistic linkages

The two R packages (party and rpart) used to construct regression trees differed in which variables they used to split the CH_4 and CO_2 fluxes, though often the differing properties were related to each other. The rpart regression tree for the full methane dataset split the data by total carbon twice, moisture three times, and soil textural class once (Figure 4.1a). This tree was more complicated than the methane regression tree built by the party package but was split by the same or similar variables. The only difference in variables selected by the two methods was soil textural class by rpart and % clay by party, suggesting that TC and moisture are strong predictors for methane fluxes. The regression tree found that soils with higher total carbon, less moisture, and loam or silt loam texture had the strongest methane uptake. However, some of the splits within the dataset were to partition out some of the non-significant fluxes that were measured; fluxes that were so small as to not be able to say with certainty that a flux occurred. To determine the potential drivers of the non-zero fluxes only, the data was subset and regression tree analysis undergone again. In total there were 47 significant methane fluxes measured, meaning approximately 26% of the methane fluxes measured were significant. When analyzing the subset methane dataset, the significant methane fluxes were split by moisture according to both methods as well as % vegetation cover and % clay (Figure 4.1b). The strongest methane uptake was found when moisture was low and vegetation cover was high. The party package split the full methane dataset fluxes by total carbon, moisture, and % clay (Figure A4.5). Moisture was the only predictor of the non-zero soil fluxes identified by party (Figure A4.6). Methane uptake was strongest when soil moisture was lower than 0.26 water fraction by volume (wfv) (i.e. 260mL of water per 1 L of soil).

The common factor of moisture between both regression trees reinforces the importance of moisture on driving methane fluxes in alpine soils. The relationship between moisture and the non-zero methane fluxes differed between sites with alpine sites and mid elevation sites in the first transect having strong negative relationships (R^2=0.398, R^2=0.250; respectively), but mid elevation sites in the second transect showing a non-significant relationship (R^2=0.008; Figure 4.3). Vegetation cover explained 21.2% of the variation in methane fluxes in sites with moisture below the threshold identified; 0.26 wfv (Figure 4.3). The interaction between soil moisture and vegetation cover drives methane uptake in our studied alpine ecosystem at Bachelor Pass.

The rpart regression tree for the CO_2 fluxes split the data by % vegetation cover, NO_3^- concentration, and $\delta^{15}N$ (Figure 4.2). This tree was much more simplified compared to the party regression tree for CO_2. The highest CO_2 emissions were found in sites with high plant cover and high available soil nitrate. Soils more enriched in $\delta^{15}N$ also had higher CO_2 emissions than soils with lower $\delta^{15}N$ values. The party regression tree analysis for the CO_2 fluxes split the data by %

vegetation cover, soil temperature, NO_3^- concentration, and soil $\delta^{15}N$ (Figure 4.7). The analysis shows that the CO_2 fluxes were generally higher when the % vegetation cover was >22%. Of those higher CO_2 fluxes those with warmer soil conditions, more available NO_3^-, and enriched $\delta^{15}N$ (suggesting older soil development) tended to have stronger CO_2 emissions.

Soil moisture, TC, and textural class were selected as the explanatory variables in the path analysis for the full methane dataset. The analysis found a strong positive relationship between moisture and methane efflux (meaning as moisture increases methane uptake decreases), and a strong negative relationship between total carbon and methane efflux (meaning as total carbon increases methane uptake increases; Figure 4.3). The path analysis confirmed the relationships between total carbon and moisture on methane uptake. In total the path model explained two-thirds of the variance in methane fluxes based on goodness of fit ($R^2=0.673$, $p<0.001$). For the subset methane dataset the variables used in the path analysis were: moisture, % clay, % vegetation cover, total carbon, and texture class. The strongest relationship was positive between moisture and methane efflux, similar to that found for the full dataset (Figure A4.8). In total the path model explained more than two-thirds of the variance in the significant methane fluxes ($R^2=0.685$, $p<0.001$). The variables used in the path analysis for the CO_2 fluxes were: soil temperature, available nitrate, and % vegetation cover. % vegetation cover had the strongest relationship with CO_2 emissions, which was positive. In total the path model explained 67.9% of the variance in the CO_2 fluxes ($R^2=0.679$, $p<0.001$; Figure A4.9). The path analysis reaffirmed the relationship between vegetation cover and CO_2 emission.

Discussion

Recently-deglaciated soil did not uptake methane and contributed the least to CO_2 emissions. These results were expected as both CH_4 and CO_2 fluxes are directly generated and also indirectly affected by vegetation and microbial communities which have minimal presence and activity in RDG sites (Table A4.2). Methane is primarily taken up by soils via methanotrophs a group of microbes that oxidize methane for energy (Hofmann et al. 2016b). The alpine sites contained on average over two order of magnitudes greater number of PLFA's in the soil than the RDG sites (Table A4.2). The observed increase in PLFA's increases the likelihood that the more developed soils contain methanotrophs as part of an active microbial community which will collectively enable the soil to uptake methane. Concomitantly, the low amount of PLFA's in the RDG sites means that there is lower likelihood of these sites containing methanotrophs which will not allow the soil to uptake methane. PLFA data was not able to be used in the regression tree analyses due to differences in sampling design, however, microbial abundance is related to vegetation cover and total carbon as microbes require organic carbon sources as substrate (Esperschütz et al. 2011, Streit et al. 2014), which may be why total carbon was found to be an important variable for splitting the data for both methane datasets in our regression tree analyses. The total carbon of the soil may act as a composite variable that contains influences from the plant and microbial communities. In addition to the microbial differences between the sites there is also a large difference in the % vegetation cover, where the RDG sites had at most 8% cover. CO_2 release from soils is largely due to the cellular respiration occurring from the microbes and plant roots in the soil (Gulledge and Schimel 2000). Relative to RDG soils, the large microbial mass and plant cover present in the alpine sites corresponds to much stronger CO_2 effluxes.

The comparisons between the CH_4 uptake in the elevational bands showed that the mid elevation sites had the strongest uptake of methane. Although the elevational bands had certain variations in vegetation cover (high band ranged from 22-64% in vegetation cover, while both mid and low bands averaged higher than 85%; Table 4.1), most of the differences in the methane fluxes are likely attributable to the observed differences in the moisture conditions across the three elevational bands. Methane uptake is more intense under lower moisture conditions (equivalent to higher soil aeration); the mid elevational band has a steeper slope than the high or low elevational bands which reduces soil moisture as the water runs downslope. Also, the low sites are located in a landscape position that captures moisture. Within the general area of the low sites, there are small streams of running water that drain from snow drifts upslope providing more consistent moisture availability, and hence, reduce soil aeration. The high sites had on average less vegetation cover than the other elevational bands which would have contributed both directly and indirectly to the differences in methane uptake between sites. The reduction in plant coverage could also have contributed to the differences in moisture conditions as with less coverage there are less plants to uptake moisture from the soil. There were also some snow drifts remaining near the high sites which may have added more moisture, reducing aeration, to the surrounding soil. Moisture was found by the regression tree analyses to be a strong predictor for methane uptake, especially when the vegetation coverage is similar among sites. Comparing the CO_2 fluxes between the elevational bands, the high elevation sites had the lowest CO_2 emissions compared to the mid and low bands. This is likely also due to the slight differences between the % vegetation cover in the different bands. Since vegetation adds substantially to the CO_2 fluxes according to the regression trees, likely through the interactions between vegetation, roots and microbial abundance, the limited coverage of the high elevational band likely reduced the amount of CO_2 produced and released from the soil.

The soil methane fluxes were found to be mostly driven by soil TC, moisture, texture, and % vegetation coverage by the two types of regression trees for both the full and subset methane dataset. These variables encompass physico-chemical parameters that relate to potential soil microbial abundance (Hofmann et al. 2016b), and soil porosity. Carbon, vegetation, and moisture are all important for microbial communities to thrive. Although our study focused on the physico-chemical drivers of gas fluxes, there are still some insights we can provide into the microbial drivers of soil fluxes. The soils at our sites all had negative methane fluxes, indicating methane uptake; it is important to remember that the net flux of methane is determined by the balance of methane production by methanogens and methane consumption by methanotrophs. Hofmann et al. (2016b) found that methanogenic bacteria decreased with increasing altitude and were absent in the nival zone (similar to the recently-deglaciated terrain in our study) on a mountain in Tyrol, Austria. Areas with higher plant cover and their associated effects tended to have a higher abundance of methanogens, this likely holds for our study as well based on the PLFA's from the RDG and alpine sites. However, Hofmann et al. (2016) found that methanotroph abundance did not seem to vary with altitude. If this applies in our study site, we would expect there to be methane uptake in the RDG sites and declining uptake in the later successional areas. Instead we found no uptake in the RDG sites and increasing methane uptake in areas with higher plant cover, larger soil total carbon concentration, and lower soil moisture. This suggests that there was an increase in the abundance and dominance of methanotrophs (or a decline in the number of methanogens) in the alpine site, which seems unlikely since the abundance of methanogens was found to be driven by similar properties as those that drove the overall methane fluxes in our site (Hofmann et al. 2016b). Since our study did not directly measure the abundance and activity of methanogenic and methanotrophic bacteria in association with the methane fluxes, it is difficult to come to a consensus relating to the underlying microbial drivers of methane fluxes in the alpine ecosystems in our study. Future work on the patterns of microbial communities in alpine areas and their effects on soil fluxes would be beneficial for further understanding greenhouse gas fluxes in alpine environments. To build on this, analysis of the archaeal communities as well as the bacterial communities is necessary as archaea may be more prominent in recently-deglaciated terrain (Esperschütz et al. 2011).

Moisture was found to be the most consistently strong predictor of methane uptake in our soils. Soil moisture may be a key driver of vegetation abundance and indirectly soil carbon content, however, since methane uptake was highest in soils under dry or intermediate moisture conditions, it is likely through the regulation of air-filled and water-filled pore space (WFPS) that moisture availability primarily drives methane fluxes. Methane oxidation requires oxygen, if there is a high WFPS, both atmospheric CH_4 and oxygen will not be able to find pathways for diffusion from the atmosphere into the soil and so methane cannot be consumed by methanotrophs. Likewise, upon oxidation of CH4, any resulting CO2 would also need to be exchanged between the soil air and the atmosphere. Fluctuations in soil water affects both the microbial and physical components of methane fluxes. The strong influence of moisture on methane uptake in montane and alpine soils has been previously identified in both China and the United States (Torn and Harte 1996, Wei et al. 2015). Our results support this relationship in Canada's Columbia Mountains as well, suggesting moisture dependence is a widespread factor controlling methane feedback in mountain ecosystems. Soil textural class is another physical driver of methane fluxes identified in our study. It was found that loam or silt loam soils had higher methane uptake than sandy loam soils, however, low % clay was also associated with higher uptake. The low % clay suggests that soils with more sand and silt, and therefore larger pore space on average, are likely to have more intense uptake of methane, possibly due to the ease of movement for gases in large, well connected pore spaces. Furthermore, soils enriched in clay typically retain more moisture (~ less aeration) over longer periods due to the high surface area of particles and dominance of small pores. However, the fact that loam or silt loam soils showed more intense methane uptake over sandy loam seems to suggest an opposite trend. Perhaps soils with too high sand content are a detrimental extreme as they can be poor growing media for vegetation establishment and organic matter accrual, collectively leading to an overall lower methane uptake in such coarse-textured soils.

The soil CO_2 fluxes are primarily driven by vegetation cover, but are also impacted by soil nitrate concentrations, $\delta^{15}N$, and soil temperature according to our results. Higher vegetation cover generally corresponds to higher microbial biomass as rhizospheres hold more microbial biomass than bulk soil (Berg and Smalla 2009). Microbes and plant roots both respire as they are living and composed of cells that release CO_2, which increases the amount of CO_2 released from the soil. High available nitrate in the soil may indicate the potential for high microbial and plant activities which would lead to higher CO_2 emissions as well. Enriched $\delta^{15}N$ values generally indicate older soils that have had a longer development time with more established plant and microbial communities, they may also indicate higher levels of denitrification in the soil (Bedard-Haughn et al. 2003). Temperature was also found to influence the CO_2 emissions which likely occurs through both temperature's impact on plant and microbial abundance, rate of enzymatic reactions, and through physical effects on gas movement and diffusivity. Interestingly, soil temperature was found to be the most effective determinant of CO_2 fluxes in this study whereas previous work had identified air temperature as being a more effective predictor (Parkin and Kaspar 2003). Our afternoon flux measurements were conducted near the daily peak of soil temperature which can have led to this increased sensitivity of our CO_2 fluxes to soil temperature (instead of air temperature).

We did not find an effect of time of day on the CO_2 fluxes from the alpine or transect sites. The soil at the alpine sites had a higher thermal buffer than the recently-deglaciated sites, meaning that the soil temperature varied less over the day in more developed soils. The difference in diurnal temperature variation between sites explains why time of day only affected the CO_2 fluxes at the RDG sites. The inter-site variability in soil temperature may be another reason for why soil temperature was a stronger predictor than air temperature for CO_2 emissions in our study. Measuring temperature at multiple depths (i.e. soil, surface, and air temperature) is advantageous for finding the most effective determinant of the CO_2 fluxes at a site as the best predictor may vary between locations and soil types. The difference in soil daily average, maximum, and minimum temperature at the soil surface and belowground between similar sites differing in elevation indicated qualitatively that belowground minimum temperatures may be the most important for upkeeping the differences in soil temperature at different elevations (Figure A4.10). The RDG and Saxifrage sites were different in terms of temperature which is due primarily to elevation differences between the sites. The strongest overlap in standard error of the mean for both sites tended to occur at the soil surface with the maximum daily temperature, which may also indicate that climate change is occurring through shifting maximum temperatures more than minimum temperatures (Figure A4.10). Soil moisture was not found to be a strong determinant of CO_2 emissions despite previous research suggesting it is a primary driver (Borken et al. 2003, Parkin and Kaspar 2003).

The combination of factors we measured may incorporate much of the variation that was explained by moisture in previous studies as the inclusion of multiple soil properties in our analyses likely provides more accurate results as to the specific properties driving fluxes over local timescales. Moisture may also be a stronger predictor on a larger timescale as it likely affects CO_2 emissions indirectly through vegetation and microbial communities as well as directly through diffusion through the soil. It is important to put our CO_2 fluxes results into a broader context, as we have only measured the CO_2 emissions from the soil, also referred to as soil respiration (Raich and Schlesinger 1992), and have not accounted for the influx amount of CO_2 taken up by plants via photosynthesis in each site. Hence, even though the sites with more vegetation had larger CO_2 emissions to the atmosphere, there is likely also a strong uptake of CO_2 into the plant tissues which would counterbalance or likely surpass the emissions, effectively leading to ecosystem C gains and gradual building up of soil organic matter throughout the successional stages. An evaluation of all C pools and fluxes in alpine environments as a function of succession and altitudinal gradients warrants additional investigation.

Future research should focus on how latitude affects the strength of fluxes at the same altitude, or seasonal effects on the magnitude and main drivers of soil fluxes as these questions are integral to our understanding on alpine fluxes across all temperate mountain ecosystems.

Global methane comparison

The methane uptake observed in our study is similar to previous studies on alpine grassland methane fluxes where precipitation was found to influence methane uptake (Wei et al. 2015, Chiri et al. 2017, Fu et al. 2018). Methane uptake peaked during the growing season in China and declined (though there was still an uptake) during the wet season (Fu et al. 2018). Older soils along glacier forefields had stronger uptake of methane than did recently-deglaciated (<15 years) areas in Switzerland (Chiri et al. 2017). This is consistent with our comparison between sites of different times since deglaciation, however we did not find significant uptake in the recently-deglaciated sites. The methane uptake rate observed in our study was more consistent and intense on average than that from previous studies in alpine regions (Wei et al. 2015, Chiri et al. 2017, Fu et al. 2018). The alpine zone in our study site had an average methane uptake of approximately -1.60 mg CH_4 m^{-2} hr^{-1}; by comparison, this methane influx is two orders of magnitude more intense than the next closest available study (Wei et al. 2015). Since mountains compose approximately 25% of the earth's surface and alpine areas account for 3% of mountain area, if we upscale these methane uptakes found at our study site to all alpine areas around the world, it would account for approximately -53.68 Tg CH_4 yr^{-1} from the atmosphere. This is likely an overestimation of the total contribution of alpine regions to the global methane budget as it does not account for variation and lower flux ranges across multiple alpine environments around the world or the seasonality of fluxes in these mountainous environments (as our measurements were conducted near peak growing season in the summer). Global estimation of methane sinks for alpine ecosystems ranges from 22 ±12 up to 36±23 Tg CH_4 yr^{-1} (Dutaur and Verchot 2007), indicating that alpine regions may contribute significantly to global methane budgets, although better estimations of alpine methane uptake globally are needed.

The uptake of methane in alpine areas is comparable to that of low elevation temperate grassland habitat, which also shows a net methane uptake (Mosier et al. 1991, Praeg et al. 2017). Temperature was found to be a driver of methane uptake in temperate grasslands, with the strongest uptake occurring in warmer temperatures. High temperatures can increase methane uptake in grassland soils above that of upland forest soils (Praeg et al. 2017), generally considered to be the strongest sinks of atmospheric methane.

Temperate upland forests account for a large proportion of the estimated 25-45 Tg CH_4 yr^{-1} captured from the atmosphere globally; roughly 4-10% of global methane sinks (Dutaur and Verchot 2007, Pitz and Megonigal 2017). There was also some methane consumption found in lowland forest soils, though it was weaker and less consistent than the upland forest (Gulledge and Schimel 2000). Keppler et al. (2006) stated a controversial finding that plants themselves were able to emit methane, though this was later disputed as showing that plants are able to uptake methane from water in the soil and release it via transpiration into the atmosphere, not produce it themselves (Nisbet et al. 2009); however, there is now the need to measure the contribution of methane emitted throughout plant transpiration from the soil water into the atmosphere (Pitz and Megonigal 2017). Future research should look to quantify the amount of methane exchanged via transpiration by both alpine plant species and montane tree species and their ability to offset methane sinks in these areas. As the treeline advances upslope, and the alpine plants also move into the recently-deglaciated terrain the alpine may become an even stronger sink for methane comparable to lower elevation grassland and forest soils mentioned above.

Another biome similar to alpine tundra is the arctic tundra, which is generally considered to be a small sink for methane (Nauta et al. 2015, Voigt et al. 2017). With increasing shrubification in the arctic drying soils, it may become an even greater sink (Myers-Smith et al. 2011). However, Zona et al. (2016) found that overall the Arctic may be a net source of methane, especially in the cold season due to emissions occurring in the thawed active layer that may persist for a long time under a thick snowpack. The apparent discrepancies between these reports can be due to the timing of their measurements (summer vs. winter) and the scale at which they are measured. Studies that claim the arctic to be a sink for methane focused at specific tundra sites, whereas Zona et al. (2016) measured fluxes at a much larger scale encompassing both dry tundra and tundra bogs and fens which are large sources of methane. At this scale Zona et al. (2016) essentially measured the net budget of methane in the arctic and found that the source of methane from peatlands outweighs the sinks from dry upland sites. Comparatively, methane emissions from peatlands globally account for approximately 20% of all natural emissions (Olefeldt et al. 2017). The effluxes from peatland systems are generally higher than the methane sinks we observed from the alpine sites in our study site (Pelletier et al. 2007, Olefeldt et al. 2017). The net methane budget, even including alpine habitat may still lean in favour of methane production, however with glacial recession and treeline advance the alpine is likely to experience a net surface expansion, and hence becoming a greater sink over time (although to the detriment of the habitats of certain native species in these areas), whereas with increasing ambient temperatures, many peatlands are starting to drain and dry which leads to a weakening in their methane emissions (Minke et al. 2016, Olefeldt et al. 2017). In particular in our field study, identified two spatial hot spots for strong methane uptake in alpine ecosystems: the alpine plateau and the alpine mid slope positions. Overall, as a function of the potential contribution of expanding alpine landscapes, methane production and consumption may tip in favour of consumption lessening the impact of methane on climate forcing as a negative feedback to ongoing climate change.

Tables

Table 4.1. Mean and standard error of all measured soil properties and vegetation coverage for each site type.

Site	Soil order	pH	EC	NO_3	NH_4	TN	TC
			dS m^{-1}	mg kg^{-1}	mg kg^{-1}	mg mg^{-1} %	mg mg^{-1} %
Early	Regosol	9.26±0.04	0.046±0.001	0.50±0.03	0.58±0.05	0.00±0.00	0.35±0.02
Late	Brunisol	5.44±0.18	0.023±0.004	0.38±0.03	2.78±0.76	0.43±0.06	8.20±1.94
High	Brunisol	6.24±0.54	0.031±0.013	0.37±0.04	1.26±0.18	0.39±0.25	3.26±2.03
Mid	Brunisol	5.37±0.09	0.020±0.005	0.47±0.08	2.45±0.66	0.62±0.12	5.47±1.12
Low	Brunisol	5.29±0.12	0.064±0.005	0.62±0.07	18.63±1.78	1.58±0.21	10.02±2.11

Site	C:N	$\delta^{15}N$	$\delta^{13}C$	Soil temperature	Surface Temperature	Air Temperature	Volumetric water content
		‰	‰	°C	°C	°C	w.f.v
Early	NA	NA	-5.40±0.30	17.26±0.71	16.19±0.90	19.74±1.00	0.05±0.01
Late	16.86±2.91	4.85±0.25	-24.03±1.25	20.85±0.62	28.92±0.96	22.60±0.75	0.27±0.01
High	8.96±0.45	4.82±0.95	-22.30±1.88	24.16±0.96	32.16±1.45	24.36±0.88	0.15±0.02
Mid	8.89±0.51	5.60±0.66	-24.43±0.24	27.13±0.79	45.16±1.93	24.91±0.54	0.21±0.03
Low	6.95±1.29	4.03±0.11	-27.28±1.51	27.06±0.49	40.32±1.81	24.67±0.35	0.53±0.02

Site	% clay	% silt	% sand	% vegetation cover
Early	10.0±0.4	32.9±3.9	57.1±4.0	1.8±0.9
Late	9.2±1.7	45.2±8.4	45.6±7.0	91.8±3.1
High	8.4±0.04	35.0±2.5	56.6±2.5	36.0±14.0
Mid	11.2±1.0	45.2±1.6	43.6±2.0	88.0±3.9
Low	11.8±0.5	46.1±3.9	42.1±4.2	84.8±3.5

Figures

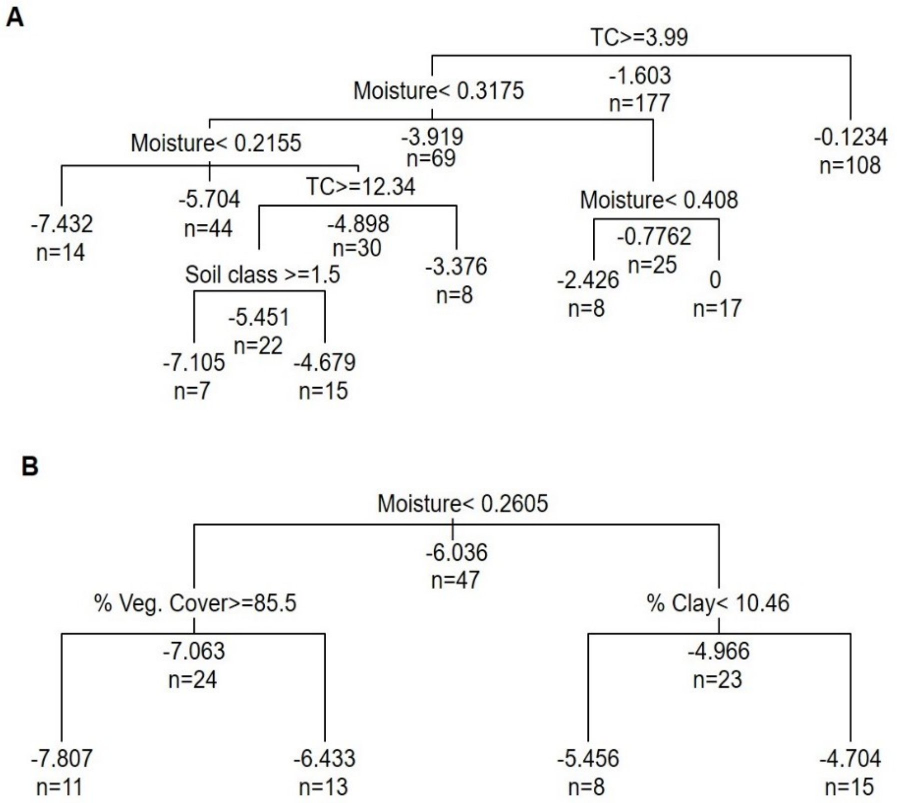

Figure 4.1. Regression trees identified the drivers of methane fluxes measured at our sites. ***A*** *full methane dataset which found moisture and total carbon (TC) to be the strongest determinants of methane uptake. Soil texture class also contributed to the data partitioning; the soil textural classes found at the sites were: sandy loam (1), loam (2), and silt loam (3). Soils with a texture class of loam or silt loam had higher methane uptake than soils with a class of sandy loam.* ***B*** *the non-zero methane dataset found moisture to be the primary split for the data, with vegetation cover and % clay also contributing to the data partitioning. Sites with low moisture and high plant coverage had the strongest methane uptake, while sites with higher moisture and high clay content had the lowest significant fluxes. The nodes are split by recursive partitioning which attempts to split the data into smaller sets so that it is easier to fit a regression to it. Each number under the node corresponds to the average methane uptake by the conditions of that node. Number of fluxes within the conditions of each terminal node are also included.*

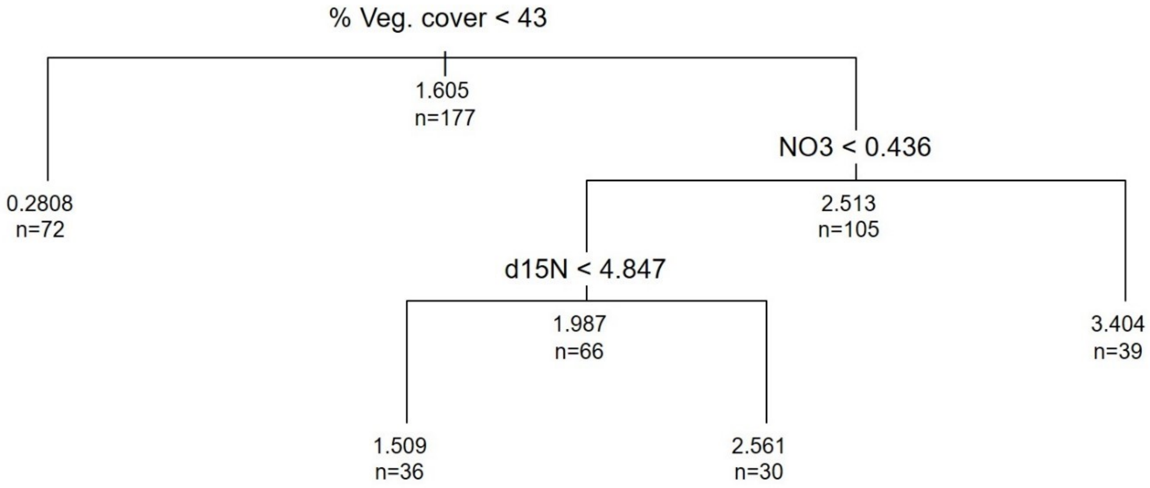

Figure 4.2. Vegetation cover, nitrate concentration, and δ15N were the drivers for the CO2 fluxes. High vegetation coverage with low nitrate concentrations contributed to the highest CO2 release from the soils. Low vegetation coverage resulted in the lowest CO2 fluxes from the sites, this would generally occur in the recently-deglaciated (RDG) sites. The nodes are split by recursive partitioning which attempts to split the data into smaller sets so that it is easier to fit a regression to it. Each number under the node corresponds to the average CO2 uptake by the conditions of that node. Number of fluxes within the conditions of each terminal node are also included.

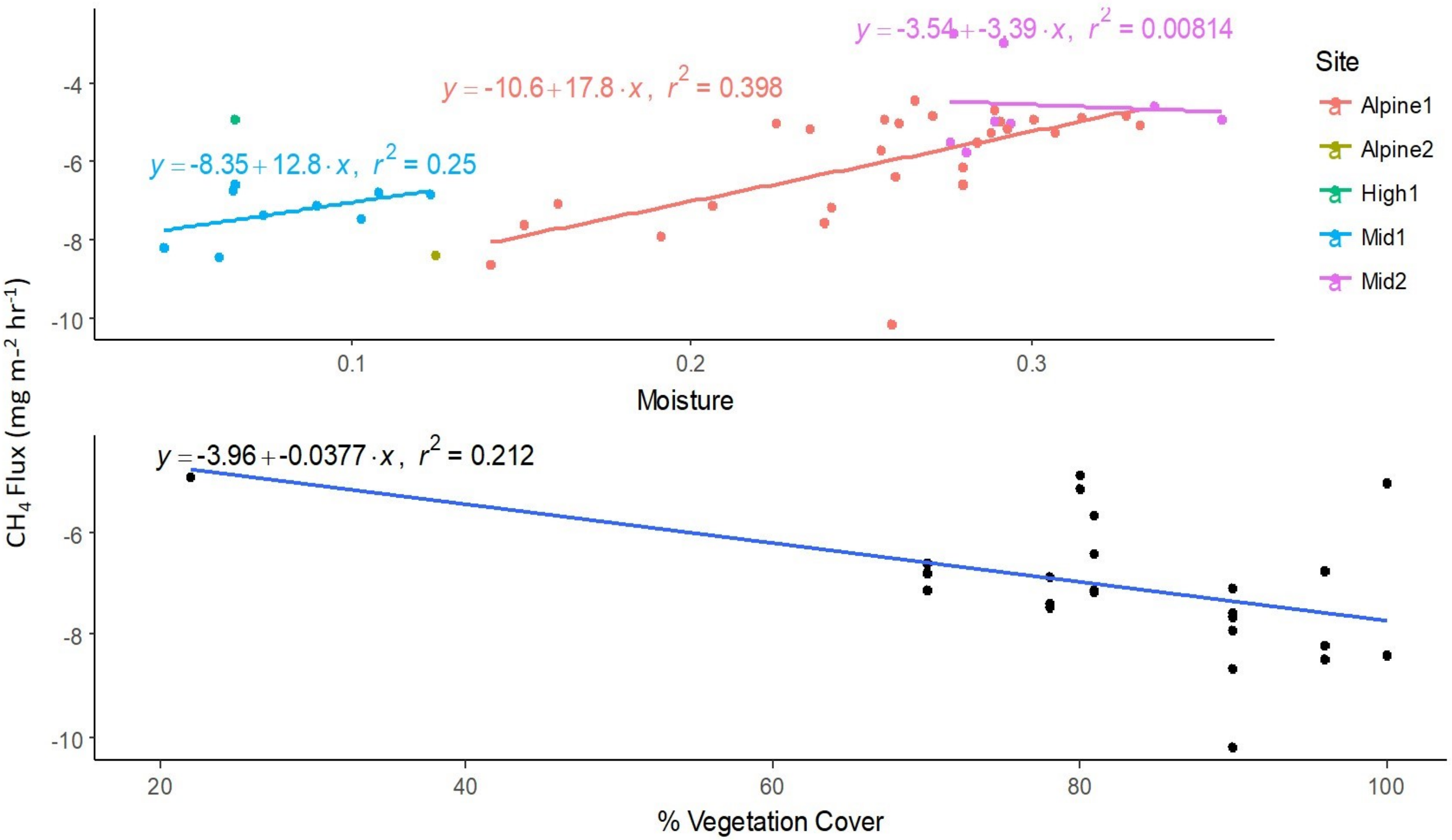

Figure 4.3. Linear relationships between methane fluxes and previously identified mechanistic drivers volumetric water content and % vegetation cover. Top panel showcases the relationships between moisture and non-zero methane fluxes for grouped by site. Sites High1 and Alpine2 only contained one non-zero methane flux and so no relationship could be determined. Lower moisture increases the strength of the methane uptake by the soil, except in site Mid2 where the moisture does not explain the variation in methane fluxes and may be better explained by other identified variables. The bottom panel shows the relationship between vegetation cover and non-zero methane fluxes when the soil moisture was below 0.2605 as identified by the regression trees. If soil moisture is not accounted for the relationship is not significant indicating an interaction between moisture and vegetation cover. Higher vegetation cover corresponds to stronger methane uptake in soils that fall within the optimal moisture range.

Appendix

Table A4.1. Average CH_4 (mg m^{-2} hr^{-1}) and CO_2 (g m^{-2} hr^{-1}) fluxes per measurement day. There was no significant difference in average fluxes for either CH_4 or CO_2 between any measurement day. Date of measurement did not influence the strength of fluxes measured and so all days were pooled together for all other analyses.

	Day 1	Day 2	Day 3
CH_4	-1.58±2.72	-1.61±2.89	-1.62±2.78
CO_2	1.77±1.51	1.70±1.60	1.35±1.25

Table A4.2. Mean and standard error of bacterial and fungal phospholipid fatty acids (PLFA) and the ratio of bacterial to fungal PLFA's (Bac:Fun) for recently-deglaciated (RDG) and alpine sites.

Site	Gram -	Gram +	Total Bacteria	Fungi	Bac:Fun
	nmol g^{-1}	nmol g^{-1}	nmol g^{-1}	nmol g^{-1}	
RDG	6.63±1.87	1.13±0.57	7.76±2.42	2.62±0.60	2.81±0.31
Alpine	435.16±51.39	696.35±84.96	1131.51±135.89	329.31±36.91	3.43±0.11

Figure A4.1. Site pictures of flux chamber bases in soil for data collection. Left panel is a typical RDG site and right panel is a typical alpine site.

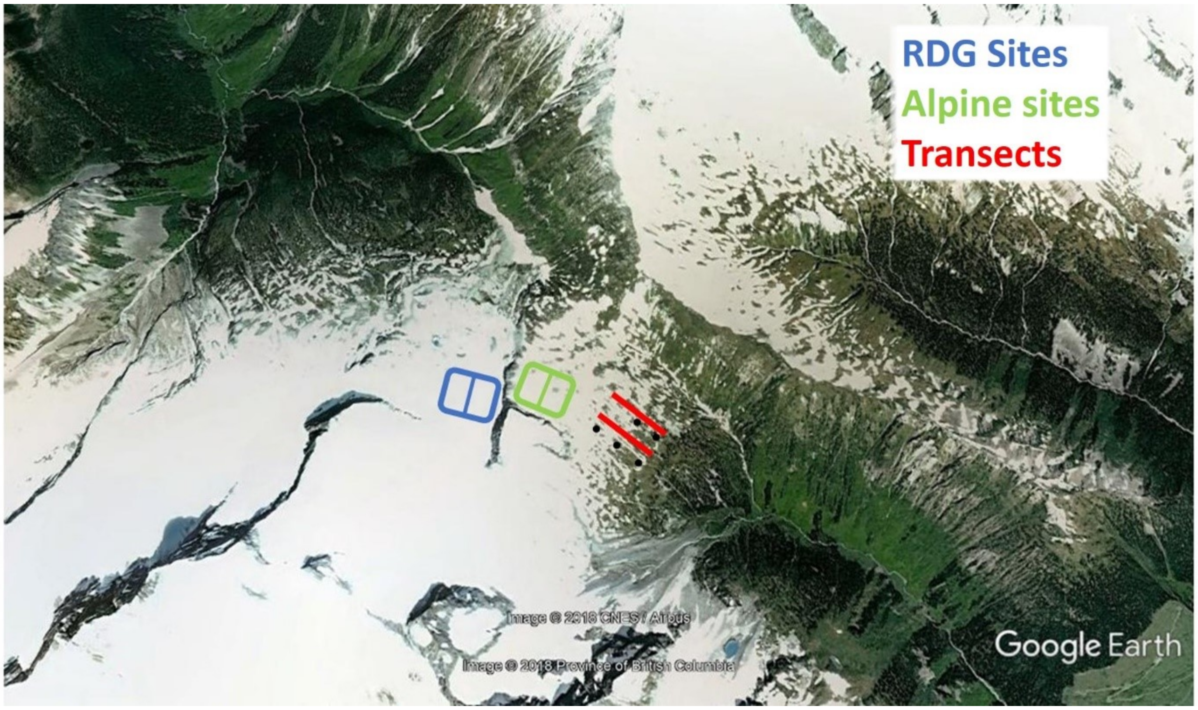

Figure A4.2. Map of Bachelor Pass displaying the approximate positions of the RDG sites (blue), alpine sites (green), and transects (red) for the chamber flux measurements. The black dots beside the transects correspond to the individual flux sites set up in three elevational bands. Image was acquired from Google Earth and the sites were added separately.

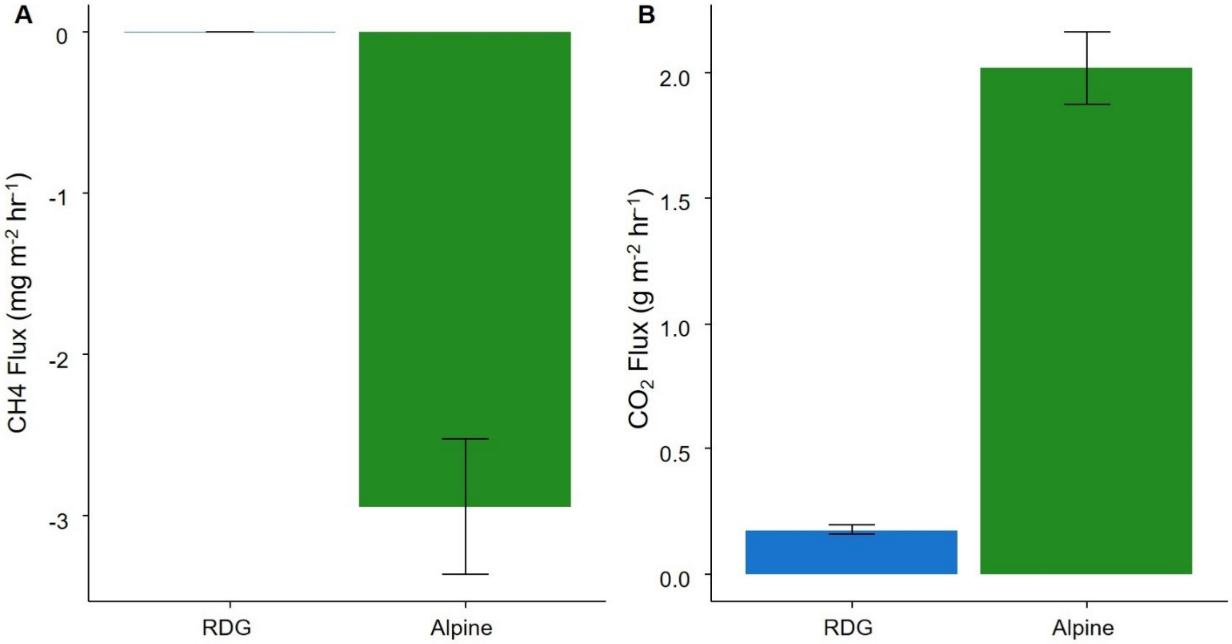

Figure A4.3. Average CH4 and CO2 fluxes in the RDG and alpine sites; respectively. A) The RDG sites lacked any significant CH4 fluxes. Alpine sites had methane uptake occur, the differences in methane fluxes was likely due to differences in vegetation and soil development. B) Alpine sites had much larger CO2 emissions than did RDG sites, likely due to differences in vegetation. Error bars represent standard error of the mean.

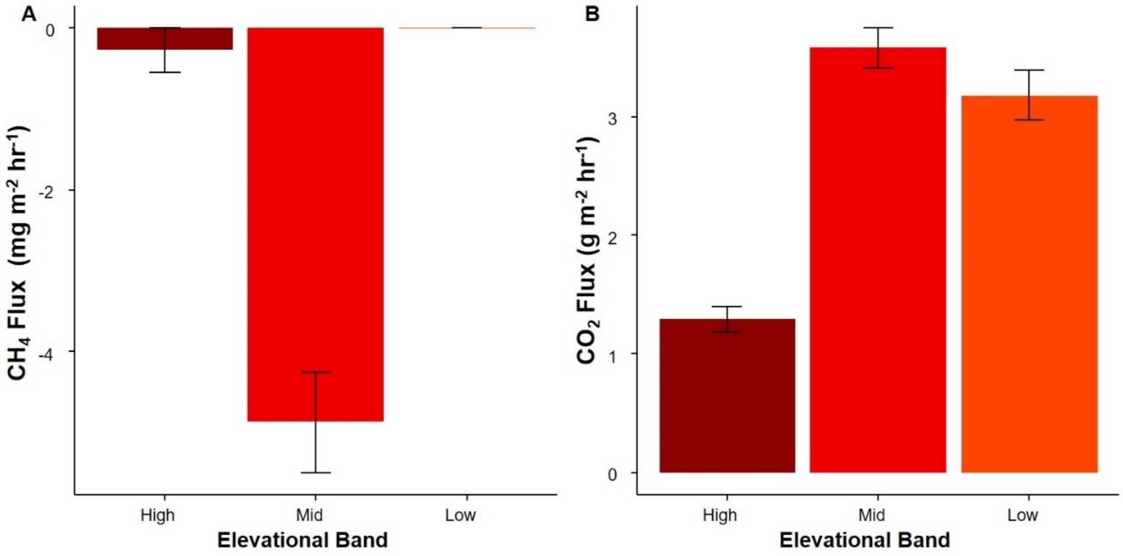

Figure A4.4. Average CH_4 and CO_2 fluxes in the high, mid, and low elevational bands; respectively. Fluxes were analyzed together from both transects according to elevational band. A) The low band sites lacked any significant CH_4 fluxes and the high band contained very few significant methane fluxes. Mid band sites had significant methane uptake, the difference between the elevational bands is likely driven by a difference in moisture dynamics. B) High elevational band sites had significantly lower CO_2 emissions than the mid or low band as they had lower vegetation cover on average. Error bars represent standard error of the mean.

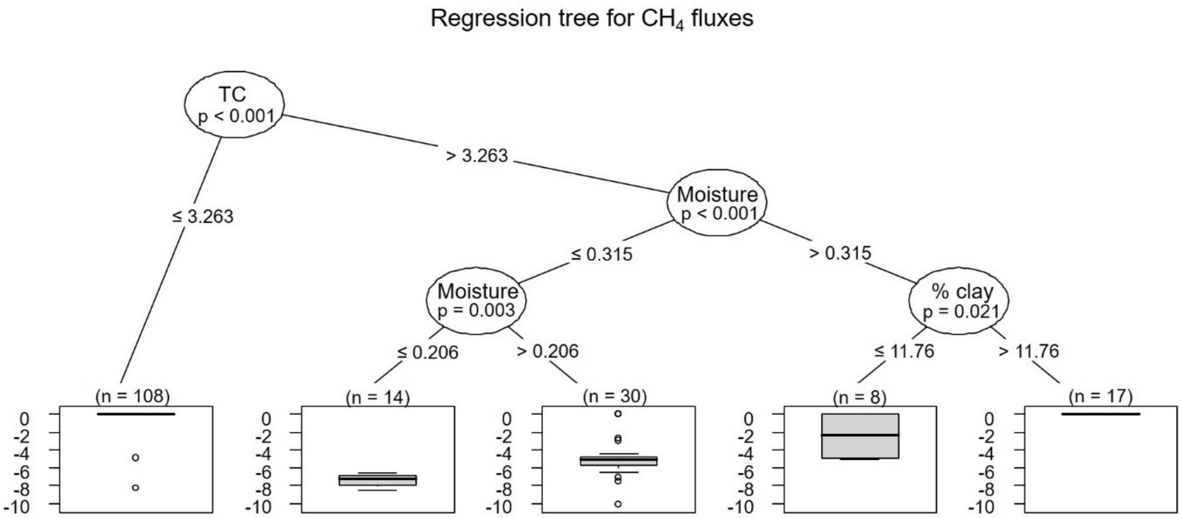

Figure A4.5. Regression tree analysis for the full methane dataset. The nodes are split by recursive partitioning which attempts to split the data into smaller sets so that it is easier to fit a regression to it. Moisture was found to be very influential for methane fluxes, while total organic carbon (TC) and % clay were useful variables for determining sites that actually contained significant methane fluxes.

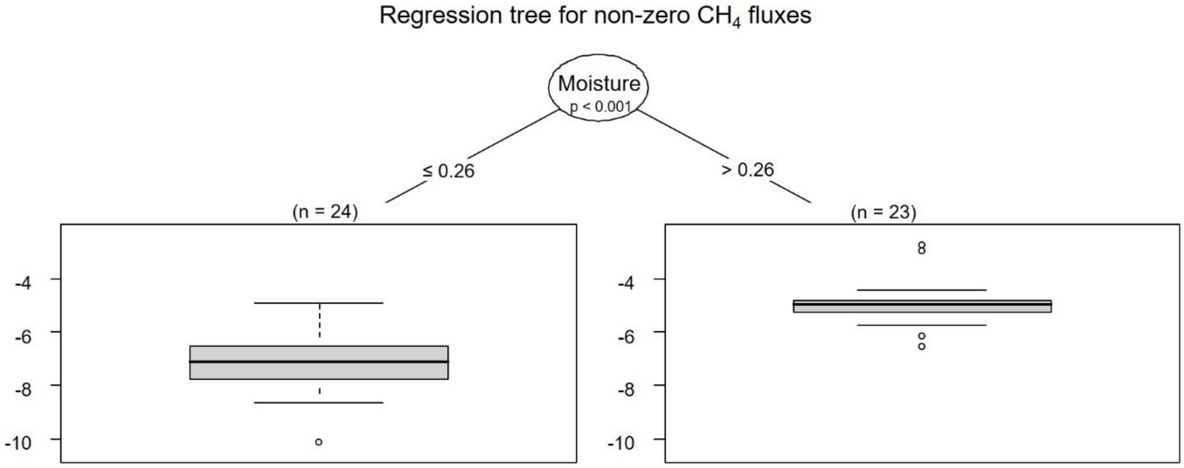

Figure A4.6. Regression tree analysis for the subset methane dataset containing non-zero fluxes only. The analysis found only one split for the data the corresponds to soil moisture. When total organic carbon (TC) and %clay of sites is similar (likely due to vegetation cover and composition) methane uptake is driven by moisture completely. The fluxes in each branch of the split were then visualized with boxplots showing the range of the methane fluxes.

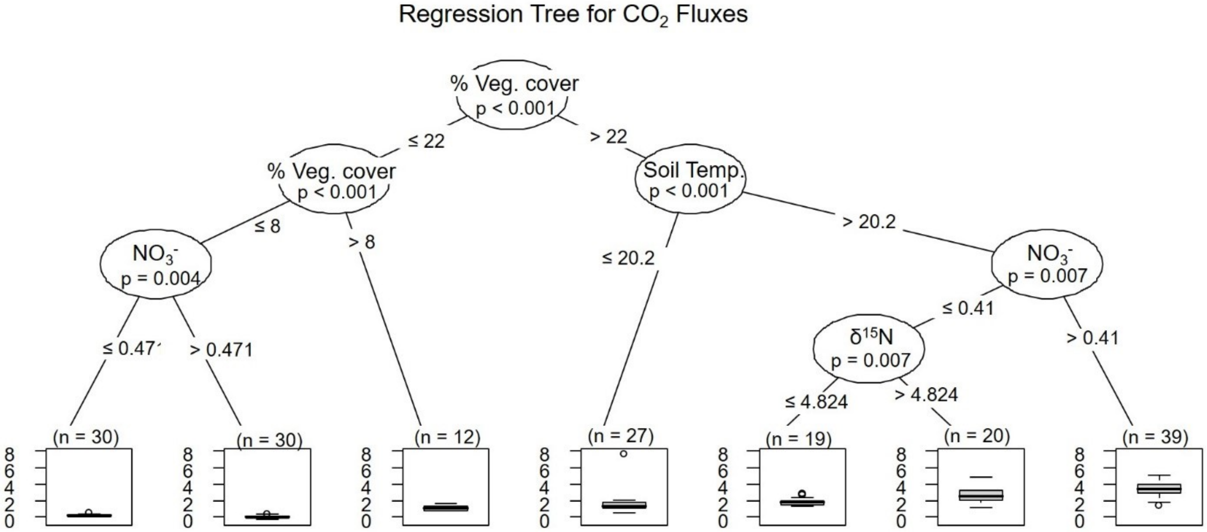

Figure A4.7. Regression tree analysis for the CO_2 fluxes. Vegetation cover was the primary split for the data where high cover corresponded to higher CO_2 emissions. Higher soil temperatures and more available NO_3^- also corresponded to higher emissions, though only if the vegetation cover was also high. Higher $\delta^{15}N$ values also contributed to higher CO_2 emissions, which may suggest that more developed soil that has been around longer will have larger CO_2 emissions.

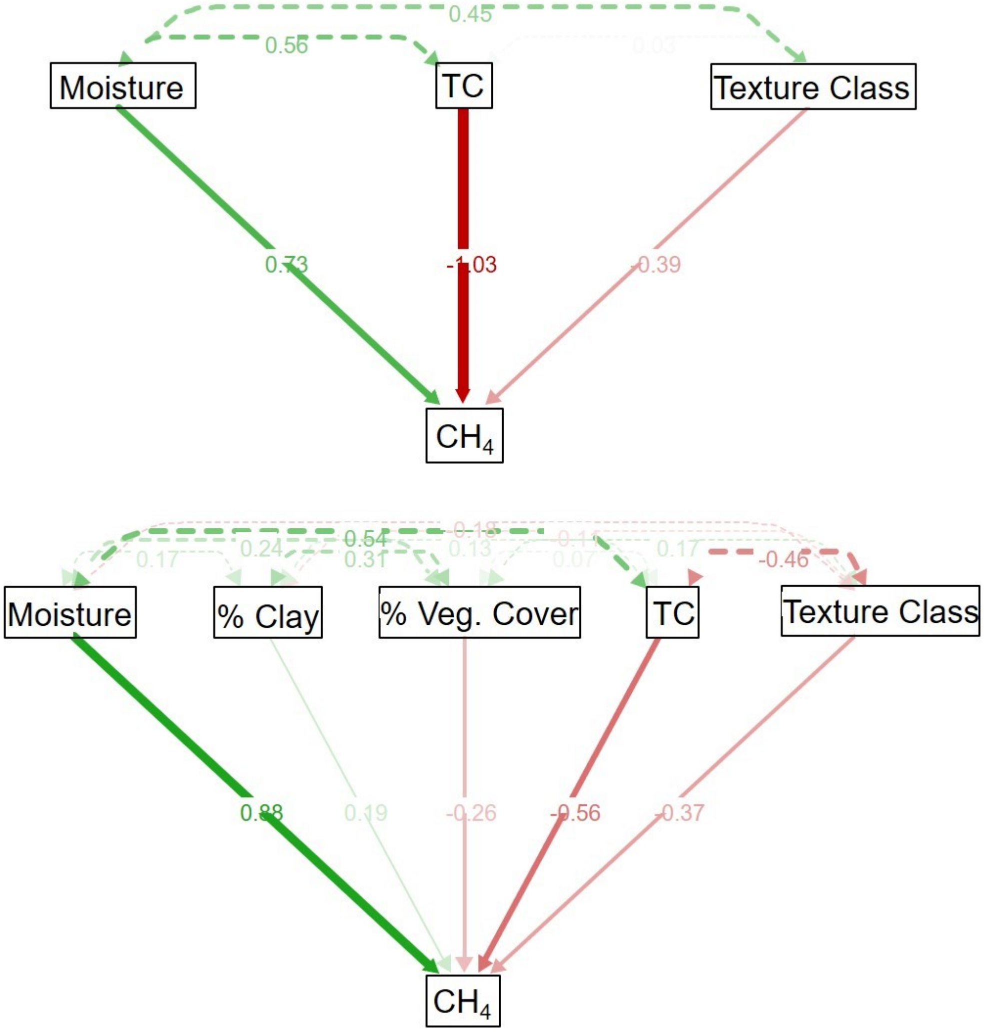

Figure A4.8. Path analysis diagrams for the full methane dataset and the non-zero methane dataset. Total carbon (TC) and moisture were the most effective drivers of methane uptake for both datasets. Moisture has a positive relationship with methane fluxes where, as moisture increases, there is a smaller methane uptake (closer to 0 or no flux). Total carbon shows the opposite trend where more carbon relates to stronger methane uptake. Soil textural class, % clay, and vegetation cover also contributed to the non-zero fluxes. Each arrow depicts the relationship between the variables, green arrows correspond to positive relationships and red arrows are negative relationships.

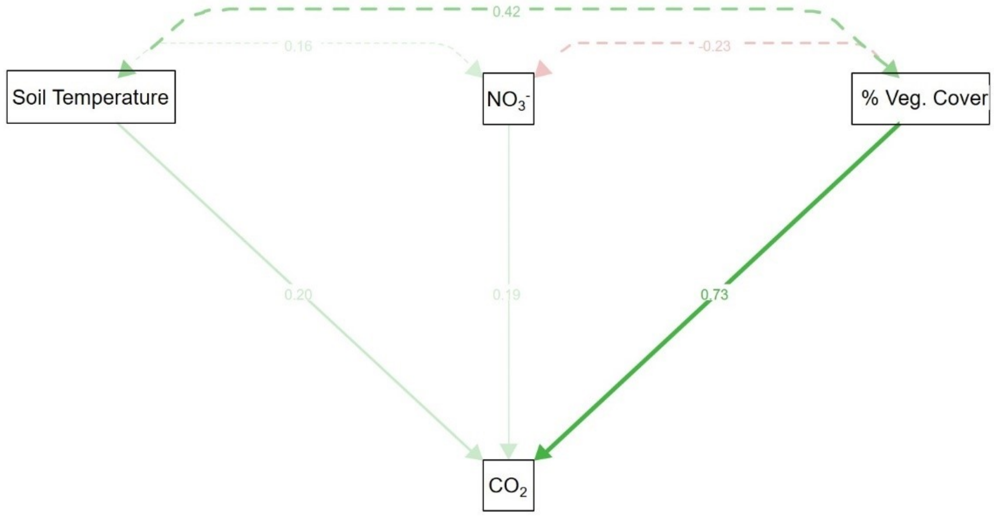

Figure A4.9. Path analysis diagram for the CO_2 dataset. Vegetation cover is the strongest driver of CO_2 emissions in the alpine. The more vegetation is available the more CO_2 is respired from both the roots and associated microbes leading to higher emissions. Each arrow depicts the relationship between the variables, green arrows correspond to positive relationships and red arrows are negative relationships.

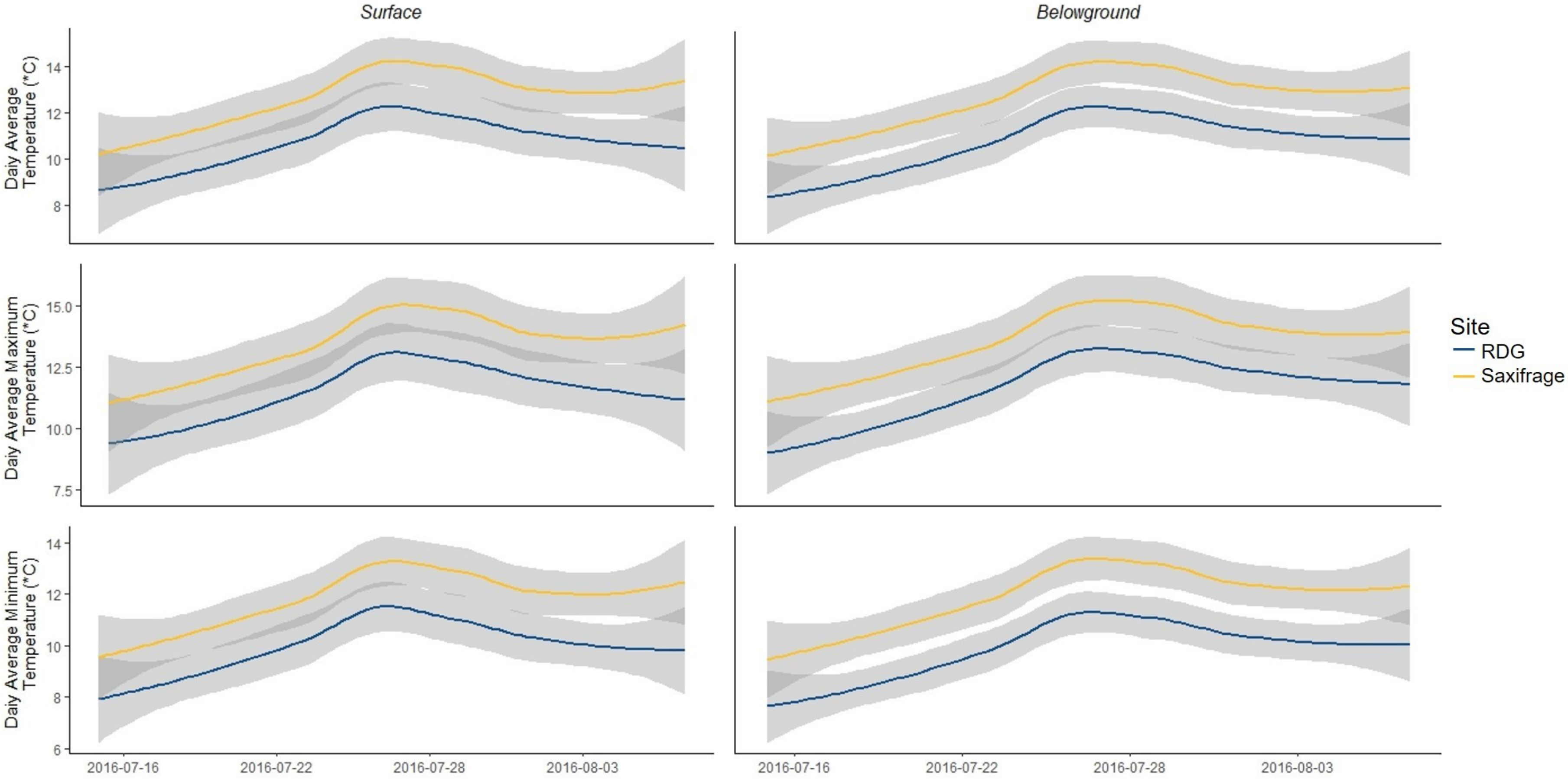

Figure A4.10. Daily average, minimum, and maximum temperatures recorded at both surface and belowground (5 cm depth) in recently-deglaciated (RDG) and Saxifrage sites. RDG sites (2126 m a.s.l) are located just below the Easy glacier and Saxifrage sites are located farther downslope closer to the valley bottom (2072 m a.s.l). Both sites have similar soil and vegetation, the main difference is elevation, therefore, the comparison mainly indicates the temperature differences due to elevation. Grey shading indicates standard error confidence intervals. There is more overlap in temperature at soil surface than belowground, and the least amount of overlap occurs in belowground minimum temperatures indicating that this level may be the most important for maintaining differences due to soil temperature along an elevational gradient.

Chapter 5

Conclusion

Mountain ecosystems are warming at an accelerated rate compared to lowland regions in many parts of the world. This has been termed "elevation-dependent warming" (EDW) and implicates the alpine as being an area of special concern for climate change, due to its high elevation and high occurrence of specialized species (Körner 2003, Nagy et al. 2003, Rangwala and Miller 2012, Pepin et al. 2015). There is some debate as to whether EDW occurs at all mountain regions and the degree to which climate warming is being accelerated, however, it seems to be consistent in temperate regions (Rangwala and Miller 2012). Accurate observations of current alpine plant and soil property distributions are essential to understand how these ecosystems will be impacted by climate change in the future. Two major consequences of climate change in mountains are shifts in ecotone boundaries, namely treeline and glacial extent (Holtmeier and Broll 2007, Rabatel et al. 2013, Chersich et al. 2015). Many studies have found that alpine treelines are either moving upslope (treeline advance) or becoming denser below the existing treeline (infilling), which has consequences on the herbaceous alpine vegetation that may get displaced as a result (Holtmeier and Broll 2007, Camarero et al. 2017).

Glaciers are also receding on average in alpine ecosystems which exposes new terrain largely devoid of life and nutrients to colonization by pioneer species starting primary succession. The degree to which alpine ecosystems will be altered by climate change depends on the potential feedback effects on greenhouse gas concentrations in the atmosphere from soils (Torn and Harte 1996, Saleska et al. 1999, Oertel et al. 2016). CH_4 and CO_2 are two major greenhouse gases (GHG's) that are transformed in the soil and exchanged with the atmosphere. The amount of GHG's released into the atmosphere determines the strength of continued climate change (Crowley 2000, Oertel et al. 2016). Understanding the magnitude and mechanistic drivers of these soil gas fluxes is imperative to determine the input from alpine regions in global climate models and the impact on continued climate change in these areas. Ultimately, proper understanding of alpine plant and soil dynamics currently and potentially in the future is essential to effectively protect and conserve these vital regions.

Characterization of current vegetation community composition along an elevational gradient in Bachelor Pass found that edaphic and topographic variables are more influential than elevation itself at local scales. Change in vegetation community composition across the treeline ecotone from montane forest to alpine grassland is impacted by soil properties more than elevation. The major driving mechanisms of plant community composition are not linear along an elevational gradient but are more influenced by local topography and plant-soil feedbacks. According to NMDS ordinations and CCA, EC, C:N, and $\delta^{15}N$ were the strongest predictors for plant community composition along the transects. Generally, this means that ion concentrations, nutrient status, and balance of N mineralization and nitrification in the soil are the primary determinants of the community at a given site. Changes in these variables due to climate change are likely to have a large impact on alpine plant communities in temperate North American mountains. As the magnitude and distribution of these properties shift with climate change alpine plant communities will adjust as well, although it is not well understood how exactly alpine plants will change in response to these changing conditions and ongoing treeline advance.

Alpine plant species have the potential to grow and survive in recently-deglaciated terrain which may enable it to act as a refuge as treeline advances potentially displacing alpine species. Nitrogen addition did not significantly affect the growth or survival of any transplanted species and was not found to increase the potential for later-successional alpine species to move into recently-deglaciated terrain. The focal species studied that were able to successfully thrive in the recently-deglaciated terrain were able to do so under current soil nutrient dynamics of the area. As climate change increases nitrogen deposition in mountainous regions there is even more potential for later-successional species to move upslope into recently-deglaciated terrain. However, competition (which is not a strong force in harsh abiotic environments) and inability to germinate in these soils may still prevent species from being able to take advantage of the newly exposed recently-deglaciated terrain due to glacial retreat (Jones and del Moral 2009). Further research into the seed banks in recently-deglaciated soil and the ability of later-successional species to germinate in these areas are critical to understand how the plant composition along the gradient from recently-deglaciated terrain to alpine will change over time.

Soil methane and carbon dioxide fluxes are both stronger in alpine grassland compared to recently-deglaciated terrain. Methane uptake is stronger in mid-elevational bands likely due to slope affecting moisture dynamics in the soil, as low moisture (and therefore, increased aeration) was the most consistent predictor of methane uptake. CO_2 emissions were higher in the mid and low elevational bands compared to the high elevational band as there was more total carbon and vegetation cover in the lower elevations. As glaciers recede and treeline advances methane uptake has the potential to increase in the alpine which acts as a negative feedback effect on climate change in these regions. The methane uptake is not strong enough to offset or counterbalance the typical efflux of methane from peatlands but is comparable to grasslands and forests in lower elevations. Climate change is also increasing drought periods which may decrease methane emissions of peatlands, which is another potential negative feedback on atmospheric methane concentrations (Chimner et al. 2017, Olefeldt et al. 2017, Zhu et al. 2018).

Mountains need to be protected for many reasons, including because they act as a sink for methane. Although the trend seems to be towards increased consumption of methane as a result of some effects of climate change, there is still potential for unseen or unknown processes to reverse this trend in the face of extensive climate change. It is vital to protect these areas now to retain their benefits in terms of biodiversity, carbon storage, and methane sink capacity. Alpine vegetation has the potential to survive in recently-deglaciated terrain exposed during glacial retreat which can provide a refuge from advancing tree species. However, the ability for later-successional alpine plants to establish in these resource-depleted soils is still unknown and will affect the potential for these species to avoid competition with treeline species. Current alpine plant communities are influenced most by soil properties compared to elevation across the treeline ecotone. Climate change effects on soil physico-chemical properties will likely have the largest effects on plant community composition at local spatial scales. However, the role of facilitation vs. competition as climate change continues, and the influence of the microbial community at these scales has not been determined at this site. Since mountains are warming at an accelerated rate due to EDW it is important to understand the repercussions of climate change on these ecosystems. Mountains act as sentinels of change for comparative lowland regions, namely grasslands and forests, and as such need to be studied and protected from ongoing anthropogenic climate change.

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