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EFFECTS OF CLIMATE CHANGE AND HERBIVORY ON ALPINE PLANTS IN THE SOUTHWEST YUKON

by

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The undersigned certify that they have read, and recommend to the Faculty of Graduate Studies and Research for acceptance, a thesis entitled **EFFECTS OF CLIMATE CHANGE AND HERBIVORY ON ALPINE PLANTS IN THE SOUTHWEST YUKON** submitted by **MICHELLE A. TAIT** in partial fulfillment of the requirements for the degree of **MASTER OF SCIENCE** in **ENVIRONMENTAL BIOLOGY AND ECOLOGY**.

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Abstract

I measured apparent photosynthesis and within-season leaf demography to study the responses of several alpine tundra species to impacts of potential climate change (warming and/or nitrogen addition) and herbivory. Overall, responses were more apparent in measurements of demography than photosynthesis and little correlation was found between the two. Six years after the initiation of the climate change manipulations, species-specific responses were observed in demographic measurements for all four study species, but only one photosynthetic response was observed. Similarly, two species showed individual mechanisms for dealing with herbivory in terms of demography, but not photosynthesis. I also examined the similarity in apparent photosynthesis and stomatal density between ten species representing four common tundra growth forms. Little similarity was observed in stomatal density, but rates of apparent photosynthesis were similar in three of four groups. Overall, the responses of alpine plants to variation in climate and herbivory are largely species-specific.

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CHAPTER 1

General Introduction

Alpine Ecosystems

The alpine life zone includes all vegetation existing beyond the high altitude treeline and covers approximately 4 million km^2 or 3% of the terrestrial surface of the globe. Alpine vegetation is unique in that it is the only biogeographic unit on land with a global distribution (Körner 1999). In addition, the presence of narrow ecotones results in steep environmental gradients. This makes it possible to move across climatic life zones in a short elevational distance that occur over large latitudinal distances (Pauli et al. 2001).

Plants growing beyond the alpine treeline are faced with a number of environmental stresses including low temperatures during the growing season, low availability of nutrients (especially nitrogen) and water, low carbon dioxide and oxygen partial pressures, intense solar radiation and strong winds (Billings and Mooney 1968, Bliss 1971, Körner 1999). In addition, alpine plants in mid- to high-latitude sites are faced with a short snow-free growing season. Nevertheless, the characteristic short stature vegetation is highly specialized to survive these severe conditions and what may seem like climatic 'extremes' are simply elements of 'normal' life for these plants (Körner 1999). Alpine species are able to thrive in their environments because they are well adapted to life under conditions that are limiting to other plant species. When these factors are no longer limiting, other species have the potential to out-compete and replace the established alpine vegetation (Körner 1999).

Due to similarities in the vegetation, areas beyond arctic and alpine treeline are often classified as 'tundra' and studies from both arctic and alpine sites are used to make general conclusions about 'tundra' ecosystems. A low daily mean temperature during the growing season is a common limiting factor for both types of tundra ecosystems, however, many differences between the two ecosystems exist. For example, low CO_2 partial pressures and strong winds are important limiting factors in alpine sites. Arctic sites have a permafrost layer that limits soil depth but that keeps the soil layer moister than most alpine soils. Additionally, arctic soils are generally more nutrient-limited than alpine soils (Billings and Mooney 1968, Bliss 1971). It is more difficult to differentiate between arctic and alpine vegetation at higher latitudes, and sub-arctic alpine vegetation is more closely related to arctic vegetation than to plants in more southern alpine areas (Bliss 1981). Both latitudinal and altitudinal gradients must be taken into consideration when making comparisons or predictions about arctic and alpine sites.

Climate Change

Temperature increases of between 1.4° and 5.8°C are predicted by the end of this century (IPCC 2001). Along with rising temperatures, other anticipated changes include increased growing season length and increased nitrogen supply (Körner 1999). Arctic and high latitude alpine ecosystems are expected to show the effects of climate change most strongly (Maxwell 1992, Lucht et al. 2002). In addition, the large stores of carbon reserves that are contained in tundra systems could be affected by changes in climate. A shift in the balance between photosynthesis and respiration could either change these systems from carbon sinks to sources or strengthen source activity (Oechel et al. 1993).

For example, studies show that moist tundra ecosystems in the Arctic have switched from CO_2 sinks to sources over the past 25 years (Oechel et al. 1993, 1995; Jones et al. 1998).

The anticipated changes seem minimal compared to the natural climatic variation that alpine plants experience, including fluctuating daily temperatures and variable growing season length. However, persistent changes in climate have the potential to affect alpine systems. Upslope migration of alpine species may occur as species become capable of invading areas that are currently inhospitable.

A study of migration in the Austrian Alps suggests that a considerable time lag exists between changes in climate and alpine vegetation responses. The mean air temperature has increased 0.5-0.7°C in the area over the past century and while upward migration has been observed in some sites, the rates are far below what would be expected given the changes in temperature that have occurred (Grabherr et al. 1995). Several inherent features have the ability to dampen or buffer the response of alpine vegetation to climate change including preformation of vegetative buds, reduced cell numbers and a high biomass allocation to belowground organs (Bowman 2000). Shorter-term climatic changes are predicted to cause a shift in community structure. How communities will respond depends greatly on abiotic conditions and initial community composition.

Studies by the International Tundra Experiment (ITEX), an international effort investigating the effects of global warming on alpine and arctic tundra plant species at sites around the world, have shown only a few general trends. Rather, most responses appear to be largely both species- and site-specific (Henry and Molau 1997, Arft et al. 1999). As well, there is a large difference between short-term and long-term effects in

experimental studies (e.g. Shaver et al. 2001). Both spatial and temporal variation must be taken into consideration when making predictions about vegetation responses to changes in climate.

Herbivory

Plants can deal with herbivory through deterrence and/or tolerance mechanisms. Herbivore defense usually involves reducing the palatability and/or digestibility of the plant material, using structures such as thorns, and secondary defense chemicals such as alkaloids (Henry and Svoboda 1994). Despite low nutrient soils, low temperatures and low production, arctic and alpine tundra systems may carry a high biomass of herbivores (Batzli et al. 1981). Several studies (Henry and Svoboda 1994, Jefferies et al. 1994, Diemer 1996, McIntire 1999, among others) suggest that some arctic and alpine plants are able to withstand carbon losses due to herbivory without negative consequences for plant vigor or reproduction, indicating that mechanisms of tolerance have been developed. Furthermore, herbivores in alpine areas have been found to have positive effects on biomass production, species richness and nutrient cycling (Körner 1999). Some of the short-term influences of herbivory on vegetation communities include slowing the rate of successional processes, increasing the rate of plant succession, and creating a vegetational mosaic (Jefferies et al. 1994).

Different growth forms have various mechanisms for coping with herbivory. For slow growing species, such as evergreens, the use of secondary defense chemicals is common as regrowth following defoliation is limited. Secondary defense chemicals are more abundant in evergreen leaves than in faster growing deciduous leaves. For graminoid species, meristematic growth can continue following a grazing event due to a

basal/intercalary meristem. For dicotyledonous plants, however, destruction of the apical meristem limits new growth within that growing season (Jefferies et al. 1994).

Interactions Between Climate Change and Herbivory

Predicted changes in climate have the potential to have large effects on the interactions between plants and herbivores (Ayres 1993). An adequate, palatable food source is one of the key concerns for small non-migrating herbivores (McIntire 1999) and the predicted changes in climate could have an impact on these food sources. In the long-term, upslope migration of treeline could reduce the land cover of alpine ecosystems and result in local extinctions of both plant and animal species. Which species survive will be a result of both dispersal mechanisms and the obstacles facing migration. Sedentary mammals are predicted to be most at risk (Murphy and Weiss 1992).

Over the short-term an increase in growing season length could have positive effects on alpine species (Murphy and Weiss 1992). Species-specific responses predicted for changes in climate will result in a change in the dominance relationships among plant species (Körner 1999). A change in vegetation composition could have a profound impact on herbivores depending on the consequences for their preferred food source. Additionally, proposed changes have the potential to alter the tissue quality of plant species. Warming and nutrient addition experiments suggest that the tissue quality of some plant species will decrease (e.g. Welker et al 1993, Schäppi and Körner 1997, Walsh et al 1997, Welker et al 1997, Suzuki and Kudo 2000, Tolvanen and Henry 2001). It has been predicted that a decrease in tissue quality will be accompanied by an increase in herbivory (Körner 1999). An increase in C:N could also result in an increase in

defensive carbon-based secondary metabolites (Ayres 1993). Others suggest that increased nutrient availability and shading, due to increased cloud cover, will increase palatability (e.g. Larsson et al 1986, Bryant et al 1987). Temperature increases are predicted to increase leaf developmental rates, thus decreasing the amount of time that young high quality forage is available for herbivores, potentially leading to a decrease in breeding success (Ayres 1993).

Study Site

This study was conducted in the Ruby Range Mountains (61°13'N, 138°16'W), southwestern Yukon Territory, Canada (Fig. 1-1). The site is classified as a sub-arctic alpine tundra environment (Price 1971). The mountains are geologically old and during the last glaciation were part of the southern arm of the Beringian intermittently ice-free landmass (Hughes et al 1968). The site consists of continuous alpine tundra vegetation and over one hundred plant species have been identified in the region, with *Dryas octopetala* L., *Salix polaris* Wahlenb., *S. reticulata* L. and *Carex consimilis* Holm being the dominant species (Price 1971, McIntire 1999). The snow-free growing season is less than 90 days and the site has low nutrient soils, characteristic of alpine sites. The altitude of the site is 1700-2100m, approximately 600 m above treeline. Daily surface temperatures may fluctuate from below 0°C to over 40°C (Hik unpublished data).

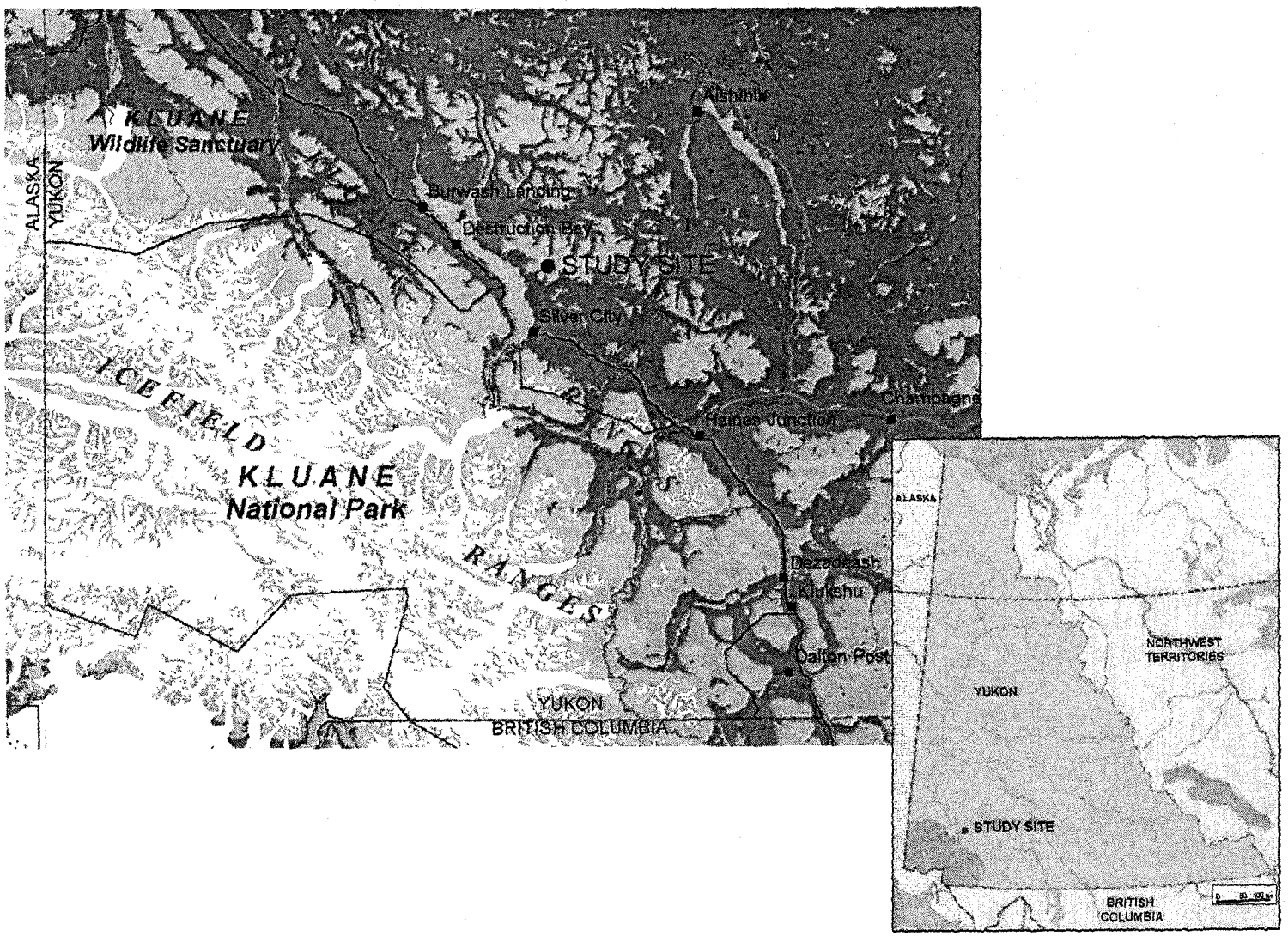

Fig. 1-1. Maps showing the location of the alpine study site in the southwest Yukon.

Study Species

Plant Species

For the climate change and herbivory experiments *Carex consimilis*, *Dryas octopetala*, *Polygonum bistorta* L. and *Salix reticulata* were focal study species. These species are common representatives of each of the tundra vascular plant forms, graminoid, semi-evergreen dwarf shrub, forb and deciduous dwarf shrub, at our study site. *C. consimilis* is a clonal sedge with rhizomatous growth (Fig. 1-2a). It is an Amphi-Beringian species and is found in central and northern Alaska and Yukon Territory and into northwestern District of Mackenzie. *D. octopetala* is a semi-evergreen or 'wintergreen' species, meaning that its leaves are functional for several growing seasons, however, chlorophyll is broken down at the end of each growing season and leaves turn brown (Fig. 1-2b). At the beginning of the next growing season these same leaves become active again. *D. octopetala* also has nitrogen fixing root nodules at some sites. *D. octopetala* has both a circumpolar distribution and is found in the alpine tundra of North America, Europe and Asia (Welker et al 1997). *P. bistorta* is an herbaceous perennial with a starchy rhizome (Fig. 1-2c). It has an Amphi-Berinigian distribution, found in Alaska and unglaciated central and northern Yukon Territory, east to the Mackenzie Delta. Other subspecies are found in Europe and Asia. *S. reticulata* is a prostrate shrub found in tundra areas as well as in forested areas within the mountains (Fig. 1-2d). It is a circumpolar arctic-alpine species, found in North America from Newfoundland and Labrador to Alaska as well as throughout the mountainous areas of the Yukon Territory, central British Columbia and western Alberta. Plant names and species descriptions used follow Cody (2000).

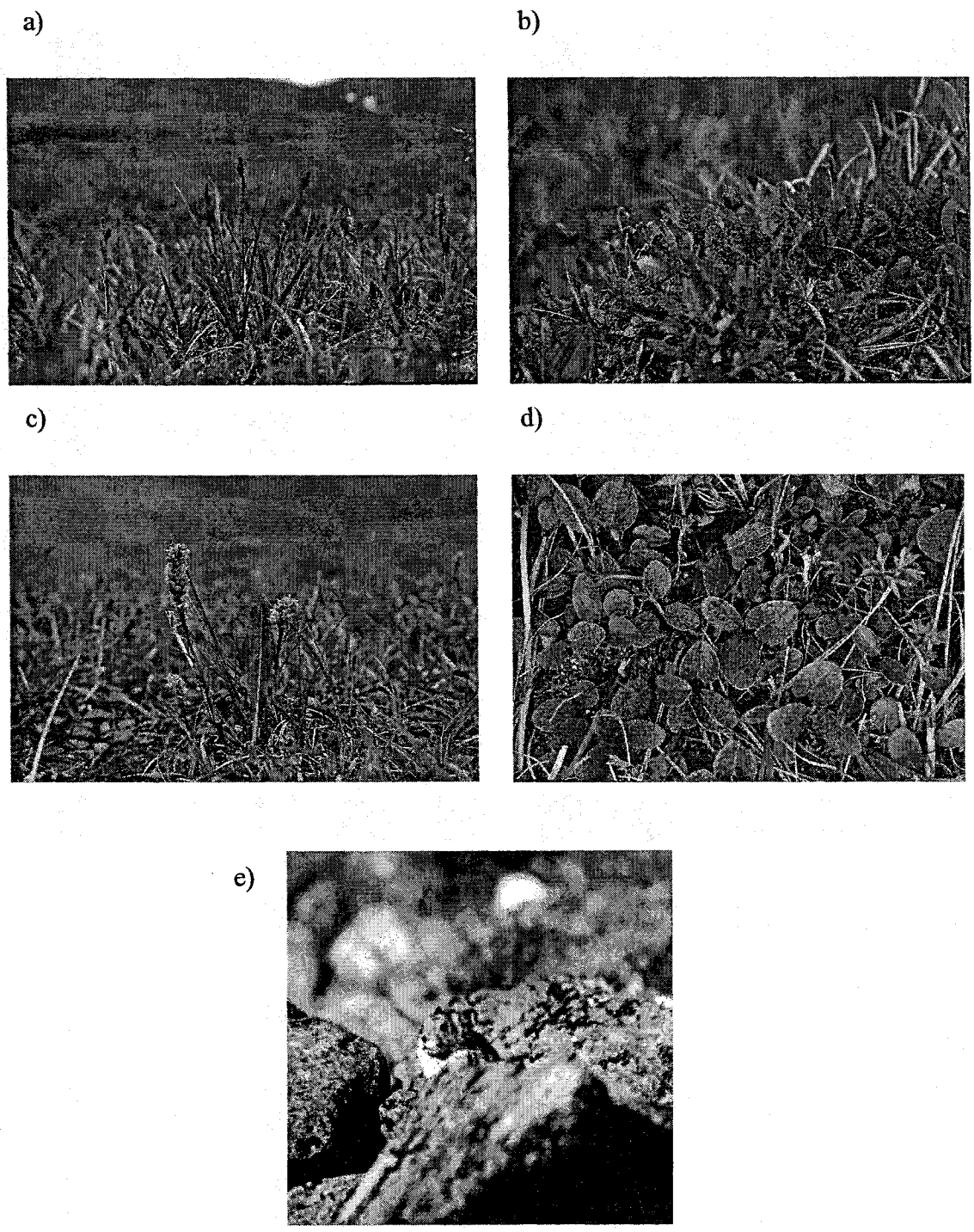

Fig. 1-2. Study species: a) *Carex consimilis* b) *Dryas octopetala*, c) *Polygonum bistorta*, d) *Salix reticulata* and e) *Ochotona collaris*.

For the species survey (Chapter 4) ten common species were used: three deciduous shrubs (*Salix arctica* Pall., *Salix polaris, Salix reticulata*), two evergreen shrubs (*Cassiope tetragona* (L.) D. Don, *Dryas octopetala*), two graminoids (*Carex consimilis, Festuca altaica* Trin.) and three herbaceous perennials (*Epilobium latifolium* L., *Petasites frigidus* (L.) Fries, *Polygonum bistorta*).

Ochotona collaris

The collared pika (*Ochotona collaris*) is a small lagomorph found in the alpine areas of south and central Yukon and Alaska (Fig. 1-2e; MacDonald and Jones 1987, Franken 2002). Collared pikas are generalist herbivores, although there is some evidence for preferences for certain vascular plant species (Andruchow 2000). Due to predation risk, pikas feed from plants adjacent to talus slopes or rock piles, where they live. The intensity of foraging increases as the summer progresses and they collect food to be stored in hay piles that will support them until the next summer. The same area, approximately three meters immediately surrounding inhabited talus, is most heavily grazed each year. The natural grazing gradient present in this system, most heavily grazed adjacent to talus, to virtually no grazing at a distance of over 6m from talus, allows for comparison of effects of grazing with minimal confounding effects. One way in which herbivores can benefit the plant communities from which they feed is via a positive feedback in nutrient cycling (Jefferies et al. 1994). However, pikas use latrines within rock piles, consequently return of nutrients to the system through urination and defecation does not benefit vegetation (*O. princeps*: Aho et al. 1998).

The intensity of grazing by pikas increases as the season progresses (McIntire 1999). Therefore, they are haying most intensively at the end of the growing season, when plant nutrient concentrations are at the lowest of the season (Chapin et al 1980). Despite being grazed at a time when plants are potentially vulnerable to damage, previous studies have shown that several plants are able to withstand this high grazing pressure at the end of the growing season (e.g. McIntire and Hik 2002). Methods for dealing with tissue loss include higher rates of production of new leaves (*Kobresia myosuroides* and *Oxytropis nigrescens*) and an end of season delay in leaf senescence (*Erigeron humilis*).

Study Objectives

In this study I attempted to determine the effects of climate change and herbivory on photosynthesis and within-season leaf demography of several alpine plant species and whether or not there is any evidence of correlation between these two sets of measurements. In addition, I looked for evidence of similarities between plant growth forms in terms of photosynthetic gas exchange and stomatal density and patterning.

My first objective was to determine potential effects of climate change on the physiology and growth of four plant species, common to the study site (Chapter 2). I measured within-season leaf demography and rates of photosynthesis in response to experimental manipulations simulating predicted climate change. Plastic greenhouses and nitrogen fertilizer were used in a factorial experimental design to simulate an increase in growing season temperature and nitrogen availability (Fig. 1-3a-c). This study was conducted five and six growing seasons after the initiation of the experiment.

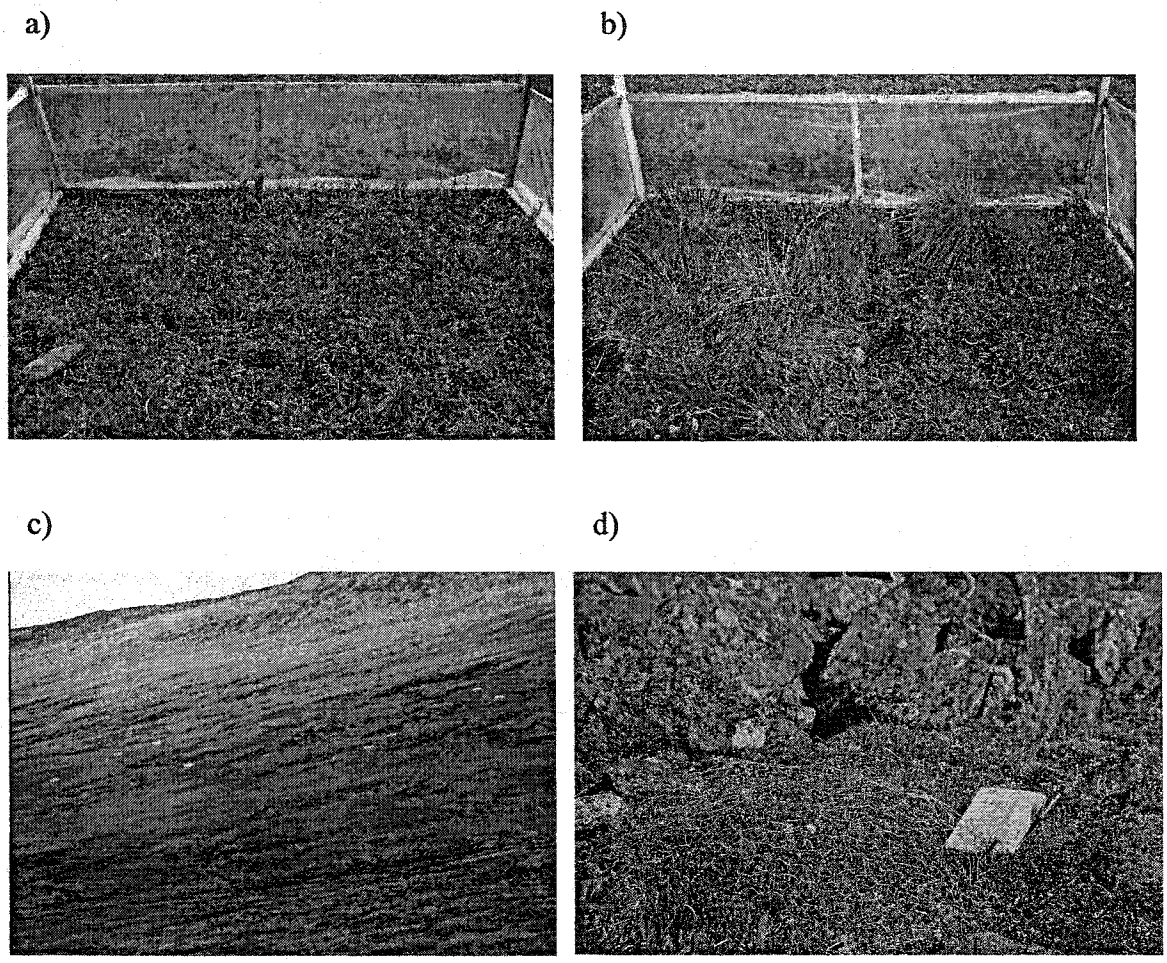

Fig. 1-3. a) Greenhouse plot without nitrogen fertilizer. b) Greenhouse plot with nitrogen fertilizer. c) Greenhouse plots on a west-facing slope. d) Herbivory exclosure with nearby control plot.

The study species were *C. consimilis*, *D. octopetala*, *P. bistorta* and *S. reticulata*. These species were chosen because: (1) they represent the four main vascular plant growth forms used in tundra ecology; graminoid, semi-evergreen shrub, forb and deciduous shrub, (2) they are dominant species at our study site and therefore important in the landscape and (3) they are important forage for resident herbivores.

My second objective was to determine the effects of both chronic (historical) and current season herbivory by a small mammal species, the collared pika (*Ochotona princeps*), on the physiology and growth of two plant species that are common pika forage (Chapter 3). Pikas graze vegetation immediately surrounding talus piles (<3m). Due to predation risk, the grazing intensity decreases sharply, moving away from talus edge. In addition, pika populations are persistent, meaning that plants close to talus probably have been grazed for many years (McIntire 1999). Using this natural grazing gradient and grazing history, I used small mesh exclosures to look for responses in *C. consimilis* and *D. octopetala* (Fig. 1-3d). Similar to Chapter 2, photosynthetic rates and within-season leaf demography were measured to look for physiological and growth responses. Because the two study species are preferred forage (Andruchow 2000) and likely experience heavy grazing pressure, I expected to observe mechanisms associated with tolerance to herbivory. By studying a graminoid and a dwarf shrub I was able to determine if different growth forms have different mechanisms for dealing with herbivory.

I chose to examine responses to both climate change and herbivory using photosynthetic rates and within-season leaf demography to determine if there was any relationship between the two sets of measurements. The number of alpine studies

examining responses at more than one scale is limited and few have found any relationships between physiological and demographic measures of production (e.g. Chapin and Shaver 1996).

Lastly, I examined the photosynthetic rates, chlorophyll fluorescence and stomatal density and patterning of ten common species, representing the four major tundra vascular plant growth forms (Chapter 4). Tundra plant species are often placed in one of four functional groups: deciduous dwarf shrub, evergreen dwarf shrub, herbaceous perennial or graminoid (e.g. Chapin et al. 1996). This grouping is useful for models predicting response to possible changes in climate as it is very difficult to include detailed information on every resident species. One of the assumptions of this grouping is that inherent physiological pathways are similar among species of the same growth form (Billings 1992). Due to these underlying physiological similarities it is thought that species of the same growth form will respond to change in a similar manner (Arft et al 1999).

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CHAPTER 2

The influence of summer warming and nitrogen addition on demography and photosynthesis of four alpine plants in the southwest Yukon

Introduction

Ongoing changes in climate are expected to be highly regional in nature, but recent assessments have concluded that high altitudes and high latitudes will experience the greatest increases in temperature and/or precipitation, particularly in the winter months (Maxwell 1992, IPCC 2001). One area where the impacts are predicted to be the greatest is in sub-arctic mountainous areas such as the southwest Yukon (Harding and McCullum 1998, Yukon State of the Environment Report 1999). In this region, the Mt Logan icecore shows a statistically significant and accelerating trend in snow accumulation from the middle of the 19th century (Holdsworth et al. 1992, Moore et al. in press). Temperatures have also increased over the past thirty years in the Yukon (Yukon State of the Environment Report 1999).

Recent reviews and comparative studies have emphasized that vegetation responses to climate change will be influenced by climatic variability, functional groups and time (Chapin et al. 1995, Henry and Molau 1997, Arft et al. 1999). Long-term data, from a variety of geographically diverse sites, on the responses of tundra plants to experimental warming as well as observational data on natural changes, are necessary for predicting how these ecosystems will respond to changes in climate, for example the Global Observation Research Initiative in Alpine Environments (GLORIA; Pauli et al. 2001) and the International Tundra Experiment (ITEX; Henry and Molau 1997). High

latitude alpine sites remain an understudied area as most research has focused on the Arctic and mid-latitude alpine areas.

Changes in precipitation and temperature may alter plant growth both directly and indirectly. Increased snow accumulation has the potential to alter C and N dynamics as reservoirs of nitrogen in the snowpack are a potentially large source of nitrogen supporting plant growth in alpine systems (Bowman 1992). Increased nitrogen availability, through increased atmospheric deposition of soluble nitrogen (Körner 1999) and increased mineralization rates due to higher temperatures, is also predicted (Melillo et al. 1990; but see Jonasson et al. 1993, Robinson et al. 1995). Global warming could alter C cycling in tundra systems, changing them from a net sink to a net source, thereby further enhancing the greenhouse effect (Welker et al. 1999).

Ecosystem responses to such changes will be dynamic and will vary both spatially and temporally. Different ecosystem processes and components respond to change at different time scales. While physiological responses may be detected almost immediately, changes in growth and population structure may take weeks or even several growing seasons to manifest themselves (Wookey et al. 1994, Shaver et al. 2000). How these short- and longer-term responses relate to one another has yet to be clearly determined. Studies that couple physiological responses with longer-term growth and community responses are required to determine how responses that act on different time scales are related. Previous studies have found that photosynthetic measurements are rarely good indicators of seasonal growth responses (Chapin and Shaver 1996), and as a result, it is often difficult to interpret the results of gas exchange studies on a larger time scale. Plants may be able to compensate for environmental change at a physiological

scale but not at a demographic scale, and the balance between these responses may reflect limitations on the responses of plants to climate change. Determining where these limitations exist is a key component to making predictions about response to changes in climate.

We report on the effects of temperature and nitrogen enhancement on the growth and photosynthetic rates of four alpine plant species in the southwest Yukon. The study was conducted five and six years after the initiation of environmental manipulations (Hik and Koh, in prep). Therefore, it is likely that the responses we measured represent adaptations to the new, experimental conditions. The objectives of this study were (1) to determine the effects of increased temperature during the growing season and/or increased nitrogen availability on the within-season leaf demography and photosynthetic rates of four species: *Carex consimilis* Holm (graminoid), *Dryas octopetala* L. (semi-evergreen shrub), *Polygonum bistorta* L. (forb) and *Salix reticulata* L. (deciduous shrub), and (2) to determine if rates of photosynthesis are good predictors of within-season leaf demography, or show changes consistent with the experimental conditions. The study species included the four main tundra vascular plant growth forms and we predicted that the greater response would be observed in response to nitrogen addition rather than increased temperatures, based on the results of previous studies in several other arctic communities (e.g. Chapin and Shaver 1985, Henry et al. 1986, Chapin et al. 1995, Robinson et al. 1998). We expected to see the largest responses in those species that are able to respond most rapidly (the forb *P. bistorta*) and the weakest responses in those species with slower leaf tissue turnover rates (the semi-evergreen shrub *D. octopetala*). In

addition, we predicted that differences in leaf demography would be consistent with variation in photosynthetic rates.

Materials and Methods

Study Site

This study was conducted in the Ruby Range Mountains in southwestern Yukon Territory, Canada (61°13'N 138°16'W, elevation 1900m a.s.l.). The site consists of continuous alpine tundra vegetation, with *Dryas octopetala*, *Salix polaris* Wahlenb., *Salix reticulata* and *Carex consimilis* being the dominant species (Price 1971, McIntire 1999).

Study Species

All of the study species were abundant at the site, large enough to measure detectable gas exchange rates using a portable IRGA, and were focal species of the International Tundra Experiment (Henry and Molau 1997, Arft et al. 1999).

The perennial *Carex consimilis* is a loosely tufted, clonal sedge with rhizomatous growth (Cody 2000, Brooker et al. 2001). It is part of the well-studied *Carex bigelowii* group, a Circumpolar Arctic-Montane species (Preston and Hill 1997). *C. consimilis* is found in a variety of habitats as it has a wide tolerance for varying climatic conditions, including areas of late snow-lie as well as on exposed ridge tops. One advantage of the rhizomatous growth form is that resources can be moved from older tillers to younger, more active tillers (Jónsdóttir and Callaghan 1989). *C. consimilis* usually reproduces vegetatively and rarely from seed and is therefore usually found in patches rather than as single individuals.

Dryas octopetala is a low, mat-forming undershrub of the Rosaceae. It has a circumpolar distribution and is found in alpine regions of North America, Europe and Asia (Cody 2000). It dominates in severe habitats with thin snow cover in winter and well-drained soil in summer. The leaves of *D. octopetala* are 'wintergreen', meaning that chlorophyll is broken down in the fall and the leaves become brown. These same leaves become photosynthetic the following spring and are usually functional for two growing seasons and remain attached for another two to four years. Having 'wintergreen', or semi-evergreen, leaves allows for early season carbon gain, growth and reproduction (Welker et al. 1997).

Polygonum bistorta is an herbaceous perennial with a thick hard rhizome (Cody 2000), found in most Arctic regions of the world. *Polygonum bistortoides*, a closely related species, is found in the Rocky Mountains. *P. bistorta* remains inactive until temperatures at the depth of its perennating bud, approximately 10cm below the surface, are 0°C. It usually sends one to three leaves from the perennating bud each growing season (Starr et al. 2000).

Salix reticulata is a prostrate deciduous shrub found in moist to dry alpine tundra. It is a circumpolar arctic-alpine species and there are three geographically distinct subspecies in North America: ssp. *reticulata*, ssp. *nivalis*, and ssp. *glabellicarpa* (Argus 1986). Ssp. *reticulata* which extends from the Arctic Archipelago southward to the Rocky Mountains of central British Columbia is found at our site (Cody 2000).

Experiment Design

In early July 1996, eight open-topped greenhouses and adjacent control plots (2.7 x 2.7 m) were established on a west-facing slope (Hik and Koh, in prep). Warming treatments were imposed using a wall of 0.2 mm plastic around a wooden frame for 6 to 10 weeks each summer. The plastic walls were removed in late August following senescence of most plants and replaced in late May or early June of the following year, after snowmelt. Mean temperatures inside the greenhouses were elevated by 1 to 2.5°C over the course of each summer (Hik and Koh, in prep). Four of the greenhouses and their respective control plots received an annual application of slow release N fertilizer (approximately $5g/m^2$), applied evenly within each plot and surrounding 1 m buffer. The snow-free growing season is less than 90 days and the site has low nutrient soils, consisting of a shallow litter layer covering deep organic peat and relatively little sand or gravel.

Leaf Demography

In late June 2001, five individuals of *C. consimilis, D. octopetala, P. bistorta* and *S. reticulata* were marked on each of the 8 greenhouse and 8 control plots using small loops of coloured wire around the base of the shoot. Plants were monitored weekly from 21 June until 9 August. Numbers of leaves, number of recent leaf births, number of recent leaf deaths, leaf length and presence or absence of flowers, were counted. New leaves were visible but not uncurled (*D. octopetala, P. bistorta* and *S. reticulata*) or less than 5mm in length (*C. consimilis*). Dead leaves were removed with scissors so that they

would not be recounted at a later date. One leaf was randomly selected and marked with black ink for leaf length measurements.

The number of leaf births and deaths were standardized to the number of leaves on each plant at each date, and are reported 'per leaf' to minimize any effects of plant size and leaf number on growth. Cumulative numbers of leaf births and leaf deaths were calculated over the entire season, as well as rates of leaf births and leaf deaths per 7-day period.

Gas Exchange Measurements

In late June 2000 and 2001, individual plants of each species were marked in each of the 8 greenhouse and 8 control plots for gas exchange measurements. To minimize the amount of handling, different plants were used for demography measurements and gas exchange measurements (2001).

Gas exchange measurements were made on a pair of plots (greenhouse and control) during a single day between 1 to 16 July 2000 and 26 June to15 July 2001. Plants were measured in a random sequence in each plot. Five plants of each species were measured on each plot during 2000. During the 2001 growing season gas exchange measurements were made on one plant of each species per plot, but these measurements (of the same plant) were made during three periods of the same day: morning (0800 to 1100), afternoon (1400 to 1700) and evening (1900 to 2200) to examine diurnal variation in gas exchange. Measurements were not made between 2200 and 0800. Measurements were also made under a variety of ambient light conditions (between 30 and 2580 μmol m^{-2} s^{-1}) to establish light response curves for the different species.

Gas exchange measurements were made using the Analytical Development Corporation (ADC) Model LCA-2 IRGA (Hoddeson, England) on fully expanded, green leaves. Due to the small size of the study species a conifer cuvette (ADC model PLC 042A) was used and measurements were made on either whole plants (*P. bistorta*) or leaf clusters (*C. consimilis*, *D. octopetala* and *S. reticulata*). Measurements of maximum apparent photosynthesis were made on days with no precipitation and full sunlight ($\geq$ 1800 μmol m^{-2} s^{-1}, except for light curve measurements). For each measurement, once the gas exchange system had stablized, the plant was left in the cuvette for a minimum of 5 minutes. During this period, the difference in CO_2 (diff) value was recorded every 30 seconds, and temperature (temp), relative humidity (rh) and photosynthetically active radiation (PAR) were recorded every minute. Measured leaves were traced onto paper in order to calculate leaf area. Apparent photosynthesis (APS) was then calculated using the formula:

APS = air flow/leaf area*(diff CO_2)*1.8,

where air flow was 150 mL/min.

Rates were determined on a μmol basis by converting the volumetric flow rate to a mass flow rate using 1.8 μg/μL to determine the weight of CO_2 at NTP and 24.5 L/mole air at NTP (Hall et al. 1993).

Equations were determined for each species to convert leaf areas to fresh mass, dry mass and chlorophyll values. This was done by measuring leaf area, fresh mass and dry mass for ten leaves of each species on each of the sixteen plots. Chlorophyll concentrations were determined using the dimethylsulfoxide (DMSO) method of extraction at 40 $\pm$ 5°C for three samples of each species per plot, using methods described

in the Appendix. A linear regression was used to determine the relationship between leaf area and either fresh mass, dry mass or chlorophyll content. Regressions were initially performed for each species for each of the four treatments. For all four study species, there were no significant differences in the relationship between leaf area and either fresh mass or dry mass for any of the treatments therefore, the same equation was used for plants from all four treatments. A separate regression was used for each of the four treatments for the relationship between leaf area and chlorophyll concentration. Rates of apparent photosynthesis were expressed on the basis of: (1) leaf area (μmol CO_2 m^{-2} s^{-1}), (2) gram fresh mass (nmol CO_2 g^{-1}FM s^{-1}), (3) gram dry mass (nmol CO_2 g^{-1}DM s^{-1}), and (4) gram chlorophyll (μmol CO_2 g^{-1} Chl s^{-1}).

Chlorophyll Fluorescence

All chlorophyll fluorescence measurements were made during the 2001 growing season using a Hansatech Fluorescence Monitoring System (FMS 2, Hansatech, King's Lynn, UK) and fully expanded, green leaves. The steady state fluorescence under the ambient light regime, F_s, was measured. A saturating light pulse was then applied to measure the maximal fluorescence yield, F_m'. A 5-sec far-red pulse was then applied and the minimum yield, F_o', recorded. The same area of leaf tissue used in the light-adapted measurements was then dark-adapted for 45 minutes using leaf clips. The minimal level of fluorescence, F_o, was measured by turning on the measuring light. Next, a saturating pulse of light was applied to measure the fluorescence maximum, F_m. The maximum quantum efficiency of PSII photochemistry was calculated as $F_v/F_m = (F_m - F_o)/F_m$, where F_v is variable fluorescence. Antennae efficiency of PSII [$F_v'/F_m' = (F_m'-F_o')/F_m'$],

quantum efficiency of PSII [$\Phi_{PSII} = (F_m'-F_s)/F_m'$], photochemical quenching co-efficient [$qP = (F_m'-F_s)/(F_m'-F_o')$] and non-photochemical quenching co-efficient [$NPQ = (F_m-F_m')/F_m'$] were also calculated.

Data Analysis

A two-way repeated measures ANOVA ($\alpha = 0.05$) was used to test the main effects of treatment (TREAT), and date (DATE) on the demographic parameters measured for each species. The influence of the main effects on leaf length, cumulative leaf births and leaf deaths and rate of leaf births and deaths was tested for each species. Leaf length data was normally distributed, however, no transformation could normalize the leaf birth and leaf death data. Because our sample sizes were quite large, the ANOVA analyses were likely robust to departures from normality (Underwood 1997). One consequence of a small number of cells with few samples in the ANOVA analyses is that formal means separation tests were not possible. Consequently, we present our results with standard error bars and emphasize the overall results of the ANOVA, rather than individual significant values.

A one-way ANOVA ($\alpha = 0.05$) was used to test the main effect of treatment (TREAT) on chlorophyll concentration and chlorophyll fluorescence values for each species. A two-way ANOVA with treatment (TREAT) and year (YEAR) as the main effects was used to test for effects on rates of APS for each species. Data met assumptions of normality and homogeneity of variance. The Least Significant Difference (LSD) test was used for post-hoc means separation tests (Carmer and Walker 1982).

Since we took more than one sample per plot, the effect of plot was nested within other main effects to minimize effects of pseudoreplication (Underwood 1997). All statistical analyses were conducted using SAS 8.1 (SAS Institute Inc. 1999).

Results

Leaf Demography

Numbers of leaf births and deaths varied significantly among species, irrespective of treatment (Fig. 2-1). By 9 August 2001, *D. octopetala* had the highest cumulative leaf births per leaf (0.8±0.03) while *S. reticulata* had the fewest (0.12±0.02). *P. bistorta* had the largest number of leaf deaths per leaf (0.36±0.04) compared to the other species (all less than 0.2).

When examined across the growing season, treatment had a significant effect on *D. octopetala* rate of leaf births and *P. bistorta* rate of leaf deaths (TREAT*DATE, Table 2-1). *D. octopetala* plants in the control and +T plots had greater rates of leaf births at the beginning of the growing season and these rates declined as the season progressed. Plants in the in the +N and +T+N plots had similar rates at the beginning of the growing season and showed no decline in rates in the second half of the growing season (Fig. 2-1). For *P. bistorta* plants in the +N and +T+N plots, greater rates of leaf deaths than plants in control and +T plots were observed on the last date of measurements (Fig. 2-1). The effect of Date was almost always significant in the RM-ANOVA (Table 2-1).

Although not statistically significant (P = 0.0788), *C. consimilis* plants in the +T+N plots had higher cumulative leaf births than the three other treatments (Table 2-1, Fig.2-1). Treatment had a significant effect on *S. reticulata* leaf length (Table 2-1, Fig.2-

2). *S. reticulata* plants in the +T+N and +N plots had the longest leaves across the growing season, while those in the control and +T plots had the shortest. Differences between treatments were present on the first date of measurements ($P = 0.009$).

Flowering

The number of plants producing flowers was very different between 2000 and 2001 (Fig.2-3). In summer 2000, flowers were found on all species in all treatments, with between 5% and 60% of plants per treatment with flowers. In contrast, in summer 2001, *D. octopetala* flowers were not observed in control, +N and +T plots and *S. reticulata* flowers were not observed in the +T plots. Overall, the number of flowering plants ranged between 0 and 25%, a significant reduction from the previous year. There were no significant treatment effects on flowering for any species in either year (Kruskal-Wallis test, all P-values ≥ 0.0925).

Gas Exchange: Methodological Considerations

In 2000 we collected gas exchange data under conditions of full sunlight, between the hours of 1100 and 1800, however in 2001 we deliberately measured plants under a variety of naturally occurring conditions. Initially, we examined relationships between light, temperature and time of day. A significant correlation was observed between PAR and temperature ($r = 0.69$, $P < 0.0001$). No significant correlations were observed between PAR and time of day ($r = -0.03$, $P = 0.523$) and temperature and time of day ($r = 0.08$, $P = 0.131$).

We then attempted to explain responses in gas exchange to different levels of PAR and time of measurement. There was no significant relationship between time of day and APS for any species. Therefore, data from any one of the three time periods, morning, afternoon and evening, were pooled for analysis of treatment differences. The light response of APS was initially examined for each treatment. While there were some treatment differences in the maximum rates of APS (see below), the PAR level at which saturation occurred was approximately the same for each treatment. For all species this occurred at levels above 200 μmol m^{-2} s^{-1}, except for *C. consimilis* for which saturation occurred around 100 μmol m^{-2} s^{-1}. Above these light intensities the slope did not deviate significantly from zero. These values were used in restricting data for later analyses.

No significant treatment differences were observed for either *C. consimilis* or *S. reticulata* for any of the chlorophyll concentration measurements (all P-values > 0.08). For *D. octopetala*, significant differences in chlorophyll *a* + *b* concentration were observed when expressed on a leaf area basis (P = 0.0378). Plants in the control plots had significantly lower concentrations of chlorophyll *a* + *b* than plants in the +N and +T+N treatments. Chlorophyll *a* + *b* concentration of plants in the +T treatment were not significantly different from any of the other treatments (Table 2-2).

For *P. bistorta* significant differences in chlorophyll *a* + *b* concentration were observed when expressed on both a fresh mass and a leaf area basis (P = 0.0454 and P = 0.0373 respectively). Plants in the +T+N treatment had a significantly higher concentration of chlorophyll *a* + *b* than plants in the controls and +T treatment. Chlorophyll *a* + *b* concentration of plants in the +N treatment were not significantly different from any of the other treatments (Table 2-2).

Gas Exchange: Species Differences

C. consimilis had the lowest rates of apparent photosynthesis (APS), *P. bistorta* and *D. octopetala* had the highest rates, and *S. reticulata* had intermediate rates (Fig. 2-4 and 2-5). This pattern was observed when expressed per m^2 leaf area, per gram fresh mass, per gram dry mass and per gram chlorophyll.

Significant treatment effects on rates of APS were observed for *P. bistorta* on a leaf area, fresh mass and dry mass basis. Significant treatment effects on rates of APS were observed for *P. bistorta* and *S. reticulata* when expressed on a chlorophyll basis. *S. reticulata* was the only species for which the effect of year was significant (Table 2-3), with slightly higher rates in 2001 on +T, +N and +T+N plots.

Rates of APS for *P. bistorta* plants in the +T treatment were significantly higher than in the three other treatments on a leaf area, fresh mass and dry mass basis (Table 2-3, Fig. 2-5). Plants in the +T plots had significantly higher rates than the +N and +T+N treatments when expressed on a chlorophyll basis. Rates of APS of plants in the controls were not significantly different from any of the treatments when expressed per gram chlorophyll (Table 2-3, Fig. 2-5).

When *S. reticulata* rates of APS were expressed on a chlorophyll basis, control plots had rates significantly higher than +N and +T+N plants and plants in +T were significantly higher than plants in +T+N (Table 2-3, Fig. 2-5).

Chlorophyll Fluorescence

No significant treatment effects were observed for any of the species for any of the measured fluorescence parameters (Table 2-4).

Discussion

Demographic and physiological responses of plants were visible five and six summers following the initiation of the temperature and nutrient manipulations. While an increase in biomass occurred in the warmed plots, the most dramatic response, at the community level, was in the nitrogen addition plots (both warmed and ambient). A decrease in the dominant shrub *D. octopetala* and an increase in graminoids, particularly grasses, resulted in a visible change in species composition (Hik and Koh, in prep).

Species Differences in Demography and Rates of APS

We predicted different demographic and photosynthetic responses from each species, reflecting their growth form. *D. octopetala* ('wintergreen' shrub) had the most leaf births per leaf by the end of the growing season, while *S. reticulata* (deciduous shrub) had the fewest, possibly because prevailing growing conditions were more favourable for *D. octopetala* (e.g, access to stored resources; Wookey et al. 1995). *P. bistorta* (forb) had the most leaf deaths by the end of the growing season compared to the other species (all similar), possibly a result of earlier senescence of *P. bistorta*.

We observed no differences between treatments in flowering. This supports the hypothesis that differences in vegetative characteristics are most likely to be observed in areas of continuous tundra vegetation (Arft et al. 1999). In addition, the difference in the number of flowers produced between the two years emphasized the role of interannual variability.

C. consimilis (graminoid), had the lowest rates of APS while *P. bistorta* and *D. octopetala* had the highest rates. *S. reticulata* had intermediate rates. The light response

curves calculated for each species demonstrated that these plants reach saturation at low light levels and are able to maintain maximal rates across a wide range of PAR values. We did not measure PAR values high enough to determine where rates of APS begin to decline. While high light levels are typical of alpine environments on clear days, cloud cover is common and restricts PAR to lower levels for much of the time. At our site, during this study, we measured PAR values ranging from 30 to 2580 μmol m^{-2} s^{-1} with a mean value of 827 $\pm$ 26 μmol m^{-2} s^{-1} and a median of 525 μmol m^{-2} s^{-1}. Reaching maximum rates at low light levels allows these species to take full advantage of lower levels of PAR. This is particularly important for plants with such a short growing season. The light response curve for *C. consimilis* is considerably lower than those of the other three species, again reflecting its low rates of APS.

Treatment Effects on Demography and Rates of APS

Our measurements of within-season leaf demography and photosynthesis suggest mechanisms for the observed changes in community composition. However, these two types of measurements were not always good predictors of one another. Species responded individually to the treatments, again possibly reflecting the different growth strategies of the growth forms.

No treatment effects on any of the chlorophyll fluorescence measurements were observed, suggesting that none of the treatments had affected the photosynthetic performance of these plants. Loik et al. (2000) observed permanent closure of PSII in the forb *Erigeron speciosus* in response to heating in a subalpine meadow. While they only observed a 2°C increase in soil temperature, they suggested that their observations are the

result of decreased soil moisture in heated plots. They also reported higher air temperatures and PAR values during their measurements than we do. The higher temperatures and light intensity could have resulted in more soil drying by the time of their measurements than had occurred at our site when we took our measurements. The fact that no photosystem damage or downregulation was observed suggests that the changes experienced in the different treatments are within the tolerance range of the study species in terms of photosynthetic performance.

C. consimilis: Graminoid

Although not significant, the increased cumulative leaf births observed for the sedge *C. consimilis* in the +T+N treatment plots suggested that both temperature and nitrogen limit the growth of this sedge at our site. If only one of the two factors was limiting then we would expect to observe a response in either the +T or the +N treatment, however plants in these plots were quite similar to those in control plots across the growing season. No treatment effects were observed for rates of APS for *C. consimilis*.

Studies of graminoids in arctic and alpine sites have found a variety of responses to increased temperature and nutrients. These responses include: increased above-ground and below-ground production and a 49% increase in leaf length under nutrient addition and an acceleration in flowering phenology, increased investment in sexual reproduction, an increase in the number of mature seeds/shoot and a 29% increase in leaf length under warmed conditions (Carlsson and Callaghan 1990, 1991; Schäppi and Körner 1996, Stenström and Jónsdóttir 1997). Different responses reflect site differences. For plants in closed alpine tundra vegetation (like our site), increased investment in sexual

reproduction may not be a valuable strategy. In arctic sites, with bare ground available for colonization, increasing sexual reproduction could be beneficial (Arft et al. 1999).

In addition to the observed effect of +T+N, it is possible that *C. consimilis* is responding to increases in temperature and/or nitrogen in a manner that was not detected by the measurements we made, such as increased tillering. In a study of the response of different tundra growth forms to fertilization, Shaver and Chapin (1980) found that NPK fertilization increased tiller mass of the sedge *Eriophorum vaginatum* by 59% while the production of new tillers was increased by >550%. Examining the response of the population of actively growing stems and tillers rather than looking for differences at the individual stem or tiller level could be more informative in the case of a tillering plant with long-lived perennial roots.

Jones (1997) also failed to find any difference in gas exchange for *Carex aquatilis stans* on warmed plots in the high Arctic. It appears that, following five and six years of the experimental manipulations, *C. consimilis* had adjusted its rates in treatment plots to match those of controls. Initially higher rates may have been observed. This is a common response in the initial year of climate and nutrient manipulations (Shaver et al. 2000). The increased leaf births for +T+N plants meant an increased area of photosynthetic tissue. Although this would result in a greater carbon gain for the whole plant this response would not be detected by our measurements of photosynthesis on a leaf area or mass basis

D. octopetala: 'Wintergreen' Shrub

A significant treatment X date interaction was observed for *D. octopetala* rate of leaf births. Plants in all four plots had similar rates of leaf births at the beginning of the growing season. For plants in the control and +T plots these rates declined as the season progressed. Plants in the +N and +T+N plots maintained similar rates of leaf births across the growing season, suggesting that nitrogen addition is delaying end of season senescence.

D. octopetala plants in the +T+N and +N treatments had significantly higher levels of chl *a* + *b* than control plants when expressed on a leaf area basis. Due to a higher chlorophyll concentration for plants in the nitrogen addition plots, we expected to observe an increase in rates of APS, on either a leaf area or mass basis. However, a higher chlorophyll concentration did not result in higher photosynthetic rates.

Suzuki and Kudo (2000) found that warming in open-top chambers resulted in higher rates of leaf survival of evergreens. Several studies of *Dryas octopetala* have found an increase in abundance, photosynthetic rates and/or biomass under warmed and/or nutrient addition treatments during the initial (up to 3) years of manipulation (Wookey et al. 1995, Welker et al. 1997, Robinson et al. 1998). However, longer-term studies of nutrient addition suggest a decrease in evergreen dwarf shrubs, including *D. octopetala* (Henry et al. 1986, Fox 1992, Chapin et al. 1995, Robinson et al. 1998). A decrease in *D. octopetala* in the nitrogen addition plots has also been observed for our site (Hik and Koh, in prep). These studies indicate that while increased nitrogen uptake can have a short-term advantage in terms of increased growth and photosynthesis, it may also result in reduced fitness in the long-term (Wookey et al. 1995, Robinson et al 1998).

Two hypotheses to account for a decrease in *D. octopetala* in nitrogen addition plots are: (1) an increase in nitrogen could inhibit winter-hardening to the point that overwinter damage kills the plants or (2) the over-supply of nitrogen is simply toxic to the plants (Henry et al. 1986). If either of these hypotheses are correct we would expect to observe either a decrease in cumulative leaf deaths in +N and +T+N plots at the end of the season (hypothesis 1) or an increase in leaf deaths in +N and +T+N plots at any point during the growing season (hypothesis 2). While we did not observe a decrease in cumulative leaf deaths, we did observe higher rates of leaf births for plants in the nitrogen addition plots later in the season when rates were declining in the control and +T plots. This pattern suggests that the higher nitrogen levels could be resulting in a delay in end of season senescence, possibly resulting in overwinter damage.

Another possibility is that *D. octopetala* is being out competed in the nitrogen addition plots. One prediction of changes in nutrient availability is that those species which are typical of nutrient rich sites will respond more strongly to nutrient addition than those that are common in nutrient poor sites (Chapin and Shaver 1985). When considering nutrient uptake it is important to consider both the response of the competitors as well as the study species (Wookey et al. 1995). The increase in graminoids in the nitrogen addition plots could result in more shading of *D. octopetala*, resulting in less carbon gain in photosynthesis (McGraw 1985).

P. bistorta: Forb

P. bistorta plants in the +N and +T+N plots had higher rates of leaf deaths than plants in control and +T plots at the end of the growing season. As well, significant

treatment effects on rates of APS were observed for *P. bistorta*. Plants in the +T plots had significantly higher rates of APS than the three other treatments on a leaf area, fresh mass and dry mass basis. *P. bistorta* plants in the +T+N treatments had higher levels of chl *a* + *b* than plants in the controls and +T treatments on both a leaf area and fresh mass basis. When expressed per gram chlorophyll, rates of APS were significantly higher in the +T plots compared to the two nitrogen addition plots, +T+N and +N. Plants in the nitrogen addition plots had the two highest chlorophyll *a* + *b* concentrations, however, this did not result in higher photosynthetic rates on a leaf area or mass basis as we predicted.

No relationship between the increased rates of APS in the +T plots and any demography measurement was observed. This suggests that something else is acting to buffer the response at the growth level (Chapin and Shaver 1996). The earlier end of season senescence for plants in the +N and +T+N plots could mean less end of season carbon gain for these plants. Measuring rates of APS across the entire growing season should be done to determine if plants in the +N and +T+N plots have higher rates of APS at any point to compensate for the earlier senescence.

An arctic study of the closely related *Polygonum viviparum* found an increase in leaf length/area but no increase in rates of APS in response to nutrient addition during the second year of manipulations. However, similar to our study, no effect of increased temperatures on vegetative parameters was observed (Wookey et al. 1994). Similarly, Starr et al. (2000) found that warming had no effect on *P. bistorta* leaf size, leaf number or photosynthetic rates. Starr et al. (2000) measured demography in the first and second years after the initiation of their experiment.

Preformation of leaves is a common feature of arctic and alpine plants (Bliss 1962, Billings and Mooney 1968). A study of *P. viviparum* found that four years are required for each leaf to reach maturity and appear aboveground (Diggle 1997). If preformation is similar in *P. bistorta* it could be that sufficient time has not yet passed to observe differences due to environmental variation, however, if a physiological response existed it should be found in these initial years of the experiment.

S. reticulata: Deciduous Shrub

For the deciduous shrub *S. reticulata*, treatment had a significant effect on leaf lengths. Plants in the +N and +T+N plots had the longest leaves across the growing season, while the plants in the control and +T plots had the shortest leaves. *S. reticulata* is the only species for which year had a significant effect on rates of APS. For the 2000 growing season, on all bases of expression, rates of APS in the control plots were significantly higher than the three other treatments. However, in 2001 rates were similar in all treatments and were similar to rates of plants in control plots in 2000. This suggests that 2001 was a more favourable year for plants in the +T, +N and +T+N plots compared to 2000 in terms of CO_2 assimilation.

As with *C. consimilis*, increasing photosynthetic area rather than photosynthetic rates per leaf area appears to be the response of *S. reticulata* to increased nitrogen, five and six years after the initiation of the experimental manipulations. Bowman and Conant (1994) also found that in the second year of a nitrogen addition experiment that the alpine willow, *Salix glauca*, responded by increasing photosynthetic area rather than rates.

General Discussion

Responses to changes in climate and nutrient availability are species-specific. Each species has a different limiting factor and therefore will show an individual response to changes in these factors (Chapin and Shaver 1985). Our results for each species are also not necessarily consistent with those presented in the literature, suggesting the importance of site-specific abiotic and biotic conditions and community composition.

Spatial and temporal variation must be taken into account when considering the results of climate manipulation and/or nutrient enhancement studies. Long-term studies provide more realistic predictions of how ecosystems will respond to potential changes. Short-term studies uncouple plant and decomposition subsystems and don't allow plants to adjust to the environmental manipulations (Wookey et al. 1995). For example, Chapin et al. (1995) and Shaver et al. (2000) found that short-term (3 yr) responses were poor predictors of longer-term (9 or 15 yr) responses.

One possible reason for the lack of response to simulated warming is that the warming acts to dry the soil, thereby limiting microbial activity, which then limits photosynthesis and growth. So, while conditions may be more favourable at the beginning of the growing season, the drying effect acts to cancel out the effects of earlier snowmelt (Harte and Shaw 1995). Water stress may be what is limiting the amount (and perhaps even the direction) of our observed responses. Moisture levels have the potential to have indirect effects as well. Shaw and Harte (2001) found very different results in nitrogen cycling between xeric and mesic sites in response to infrared heating. An initial increase in net mineralization rates was observed for the xeric site while no significant

effects of heating on any N transformation rates were observed for the mesic site. This result suggests why several studies have observed increased microbial activity with warming while others have not (e.g. Billings et al 1982, 1983 and Melillo et al 1990 compared to Jonasson et al 1993, Robinson et al 1995). In general, any comparison of results of arctic and alpine studies where the initial soil microclimate conditions were very different should be made cautiously (Shaw and Harte 2001). Another possible reason for the lack of response to warming could be that the temperature change is not large enough to stimulate any response. Temperatures can span a wide range at our alpine site, and it is possible that the imposed warming (1-2.5°C) is not substantial enough to stimulate any responses.

Overall, we observed little correlation between measurements of rates of photosynthesis and within-season leaf demography. Other studies (Wookey et al. 1994, Chapin and Shaver 1996) have also found that physiological responses are not good predictors of growth responses. Compensatory mechanisms act to buffer short-term responses as a considerable time lag exists between physiological CO_2 uptake and where it will be distributed in growth and reproduction (Chapin and Shaver 1996).

Nevertheless, five and six summers after the initiation of temperature and nitrogen manipulations at a sub-arctic alpine site, species-specific responses are clearly visible (Hik and Koh, in prep). In our study, treatment differences were more pronounced in measurements of leaf demography across the growing season compared to measurements of instantaneous CO_2 uptake. Our results suggest that warming and/or increased nitrogen have the potential to alter species composition.

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Table 2-1. Summary of F-values from RM-ANOVAs for leaf demographic characteristics measured in 2001. Main effects are treatment (TREAT) and sampling date (DATE). Significant values ($P < 0.05$) are in bold.

C. consimilis

	Cumulative leaf births		Cumulative leaf deaths		Rate of leaf births		Rate of leaf deaths		Leaf length	
	$F_{(numdf, dendf)}$	P	$F_{(numdf, dendf)}$	P	$F_{(numdf, dendf)}$	P	$F_{(numdf, dendf)}$	P	$F_{(numdf, dendf)}$	P
TREAT	$2.90_{(3,12)}$	0.0788	$0.19_{(3,12)}$	0.9009	$1.93_{(3,12)}$	0.1784	$0.33_{(3,12)}$	0.8008	$0.29_{(3,12)}$	0.8340
DATE	**$25.60_{(7,596)}$**	**<.0001**	**$25.49_{(7,596)}$**	**<.0001**	**$5.10_{(7,596)}$**	**<.0001**	**$5.80_{(7,596)}$**	**<.0001**	**$89.90_{(7,596)}$**	**<.0001**
TREAT*DATE	$1.05_{(21,596)}$	0.5294	$0.63_{(21,596)}$	0.9018	$0.88_{(21,596)}$	0.6227	$1.32_{(21,596)}$	0.1525	$0.11_{(21,596)}$	1.0000

D. octopetala

	Cumulative leaf births		Cumulative leaf deaths		Rate of leaf births		Rate of leaf deaths		Leaf length	
	$F_{(numdf, dendf)}$	P	$F_{(numdf, dendf)}$	P	$F_{(numdf, dendf)}$	P	$F_{(numdf, dendf)}$	P	$F_{(numdf, dendf)}$	P
TREAT	$0.51_{(3,12)}$	0.6808	$1.61_{(3,12)}$	0.2397	$0.93_{(3,12)}$	0.4563	$2.30_{(3,12)}$	0.1298	$0.29_{(3,12)}$	0.8298
DATE	**$97.34_{(7,596)}$**	**<.0001**	**$11.59_{(7,596)}$**	**<.0001**	$1.30_{(7,590)}$	0.2478	$1.74_{(7,592)}$	0.0965	**$4.94_{(7,586)}$**	**<.0001**
TREAT*DATE	$0.75_{(21,596)}$	0.7851	$0.62_{(21,596)}$	0.9021	**$1.58_{(21,590)}$**	**0.0497**	$0.45_{(21,592)}$	0.9846	$0.89_{(21,586)}$	0.5990

P. bistorta

	Cumulative leaf births		Cumulative leaf deaths		Rate of leaf births		Rate of leaf deaths		Leaf length	
	$F_{(numdf, dendf)}$	P	$F_{(numdf, dendf)}$	P	$F_{(numdf, dendf)}$	P	$F_{(numdf, dendf)}$	P	$F_{(numdf, dendf)}$	P
TREAT	$0.10_{(3,12)}$	0.9588	$0.53_{(3,12)}$	0.6725	$038_{(3,12)}$	0.7675	$1.38_{(3,12)}$	0.2955	$1.57_{(3,12)}$	0.2471
DATE	**$18.31_{(7,588)}$**	**<.0001**	**$33.28_{(7,588)}$**	**<.0001**	**$4.30_{(7,577)}$**	**0.0001**	**$8.71_{(7,580)}$**	**<.0001**	**$10.01_{(7,567)}$**	**<.0001**
TREAT*DATE	$0.40_{(21,588)}$	0.9223	$0.78_{(21,588)}$	0.7433	$0.83_{(21,577)}$	0.6863	**$1.60_{(21,580)}$**	**0.0433**	$0.70_{(21,567)}$	0.8313

S. reticulata

	Cumulative leaf births		Cumulative leaf deaths		Rate of leaf births		Rate of leaf deaths		Leaf length	
	$F_{(numdf, dendf)}$	P	$F_{(numdf, dendf)}$	P	$F_{(numdf, dendf)}$	P	$F_{(numdf, dendf)}$	P	$F_{(numdf, dendf)}$	P
TREAT	$0.63_{(3,12)}$	0.6068	$0.61_{(3,12)}$	0.6205	$1.23_{(3,12)}$	0.3420	$0.80_{(3,12)}$	0.5192	**$6.98_{(3,12)}$**	**0.0057**
DATE	**$2.77_{(7,596)}$**	**0.0077**	**$6.52_{(7,596)}$**	**<.0001**	$0.55_{(7,591)}$	0.7995	$1.74_{(7,593)}$	0.0972	**$6.03_{(7,583)}$**	**<.0001**
TREAT*DATE	$0.22_{(21,596)}$	0.9999	$0.32_{(21,596)}$	0.9987	$0.76_{(21,591)}$	0.7647	$0.75_{(21,593)}$	0.7835	$0.67_{(21,583)}$	0.8653

Table 2-2. Chlorophyll ($a + b$) concentration on a fresh mass (μgChl(a+b)/100mgFM) and a leaf area basis (μgChl(a+b)/cm^2) for each of the study species.

		μgChl(a+b)/100mgFM	μgChl(a+b)/cm^2
C. consimilis	Control	280 ± 10	63 ± 2
	+T	289 ± 14	65 ± 3
	+N	354 ± 47	79 ± 10
	+T+N	383 ± 51	86 ± 11
D. octopetala	Control	227 ± 22	52 ± 2
	+T	242 ± 10	61 ± 3
	+N	292 ± 17	75 ± 5
	+T+N	325 ± 33	83 ± 9
P. bistorta	Control	188 ± 10	77 ± 4
	+T	174 ± 8	71 ± 3
	+N	255 ± 34	106 ± 15
	+T+N	288 ± 28	120 ± 12
S. reticulata	Control	194 ± 11	72 ± 4
	+T	208 ± 13	77 ± 5
	+N	244 ± 23	92 ± 9
	+T+N	253 ± 72	96 ± 30

Table 2-3. Summary of F-values from ANOVAs for gas exchange measurements. Main effects are treatment (TREAT) and year. Significant values ($P < 0.05$) are in bold.

		$\mu molCO_2\ m^{-2}\ s^{-1}$		$nmolCO_2\ g^{-1}\ FM\ s^{-1}$		$nmolCO_2\ g^{-1}\ DM\ s^{-1}$		$\mu molCO_2\ g^{-1}\ Chl\ s^{-1}$	
		$F_{(numdf, dendf)}$	P	$F_{(numdf, dendf)}$	P	$F_{(numdf, dendf)}$	P	$F_{(numdf, dendf)}$	P
C. consimilis	TREAT	$2.4034_{(3,3)}$	0.2451	$2.1789_{(3,3)}$	0.2694	$2.1979_{(3,3)}$	0.2672	$0.1354_{(3,12)}$	0.9370
	YEAR	$1.6631_{(1,24)}$	0.2095	$1.6580_{(1,24)}$	0.2102	$1.6585_{(1,24)}$	0.2101	---	---
	TREAT*YEAR	$0.1064_{(3,24)}$	0.9555	$0.1145_{(3,24)}$	0.9507	$0.1138_{(3,24)}$	0.9512	---	---
D. octoeptala	TREAT	$0.2383_{(3,3)}$	0.8653	$0.2387_{(3,3)}$	0.8650	$0.2392_{(3,3)}$	0.8647	$2.559_{1(3,12)}$	0.1039
	YEAR	$0.9531_{(1,24)}$	0.3387	$1.5496_{(1,24)}$	0.2252	$1.7739_{(1,24)}$	0.1954	---	---
	TREAT*YEAR	$1.7398_{(3,24)}$	0.1856	$1.8286_{(3,24)}$	0.1689	$1.8573_{(3,24)}$	0.1638	---	---
P. bistorta	TREAT	**$11.8388_{(3,3)}$**	**0.0360**	**$10.8416_{(3,3)}$**	**0.0406**	**$18.2697_{(3,3)}$**	**0.0196**	**$6.6403_{(3,12)}$**	**0.0068**
	YEAR	$0.0190_{(1,24)}$	0.8916	$0.0116_{(1,24)}$	0.9151	$0.0678_{(1,24)}$	0.7968	---	---
	TREAT*YEAR	$0.5427_{(3,24)}$	0.6577	$0.6071_{(3,24)}$	0.6168	$0.3065_{(3,24)}$	0.8204	---	---
S. reticulata	TREAT	$0.7763_{(3,3)}$	0.5800	$0.7367_{(3,3)}$	0.5962	$0.8314_{(3,3)}$	0.5585	**$11.0250_{(3,8)}$**	**0.0033**
	YEAR	**$6.4255_{(1,16)}$**	**0.0221**	**$6.2870_{(1,16)}$**	**0.0233**	**$6.4583_{(1,16)}$**	**0.0218**	---	---
	TREAT*YEAR	**$3.3228_{(3,16)}$**	**0.0465**	$3.1723_{(3,16)}$	0.0530	**$3.4696_{(3,16)}$**	**0.0411**	---	---

Table 2-4. Chlorophyll fluorescence values for each of the study species. See methods for details.

C. consimilis

	control	+N	+T	+T+N
F_v/F_m	0.761 ± 0.021	0.766 ± 0.014	0.762 ± 0.012	0.747 ± 0.009
F_v'/F_m'	0.663 ± 0.034	0.633 ± 0.022	0.663 ± 0.032	0.686 ± 0.012
I_{PSII}	0.564 ± 0.034	0.537 ± 0.030	0.576 ± 0.039	0.612 ± 0.020
qP	0.852 ± 0.027	0.842 ± 0.026	0.863 ± 0.032	0.885 ± 0.026
NPQ	1.043 ± 0.229	0.960 ± 0.210	0.582 ± 0.210	0.510 ± 0.182

D. octopetala

	control	+N	+T	+T+N
F_v/F_m	0802 ± 0.012	0.753 ± 0.015	0.784 ± 0.015	0.762 ± 0.015
F_v'/F_m'	0.721 ± 0.019	0.707 ± 0.025	0.703 ± 0.021	0.684 ± 0.026
I_{PSII}	0.615 ± 0.036	0.625 ± 0.031	0.621 ± 0.028	0.616 ± 0.032
qP	0.852 ± 0.041	0.898 ± 0.039	0.879 ± 0.019	0.884 ± 0.024
NPQ	0.504 ± 0.119	0.541 ± 0.072	0.903 ± 0.253	0.800 ± 0.235

P. bistorta

	control	+N	+T	+T+N
F_v/F_m	0.804 ± 0.005	0.779 ± 0.010	0.797 ± 0.012	0.794 ± 0.006
F_v'/F_m'	0.749 ± 0.015	0.699 ± 0.023	0.731 ± 0.019	0.691 ± 0.033
I_{PSII}	0.673 ± 0.028	0.645 ± 0.025	0.661 ± 0.031	0.661 ± 0.029
qP	0.896 ± 0.027	0.920 ± 0.015	0.903 ± 0.029	0.907 ± 0.024
NPQ	0.574 ± 0.105	0.650 ± 0.137	0.612 ± 0.185	0.609 ± 0.190

S. reticulata

	control	+N	+T	+T+N
F_v/F_m	0.795 ± 0.010	0.747 ± 0.015	0.790 ± 0.011	0.776 ± 0.015
F_v'/F_m'	0.668 ± 0.034	0.650 ± 0.021	0.714 ± 0.048	0.684 ± 0.029
I_{PSII}	0.564 ± 0.043	0.569 ± 0.026	0.614 ± 0.041	0.612 ± 0.038
qP	0.821 ± 0.032	0.869 ± 0.019	0.906 ± 0.019	0.864 ± 0.032
NPQ	1.186 ± 0.229	0.861 ± 0.162	0.934 ± 0.359	1.197 ± 0.275

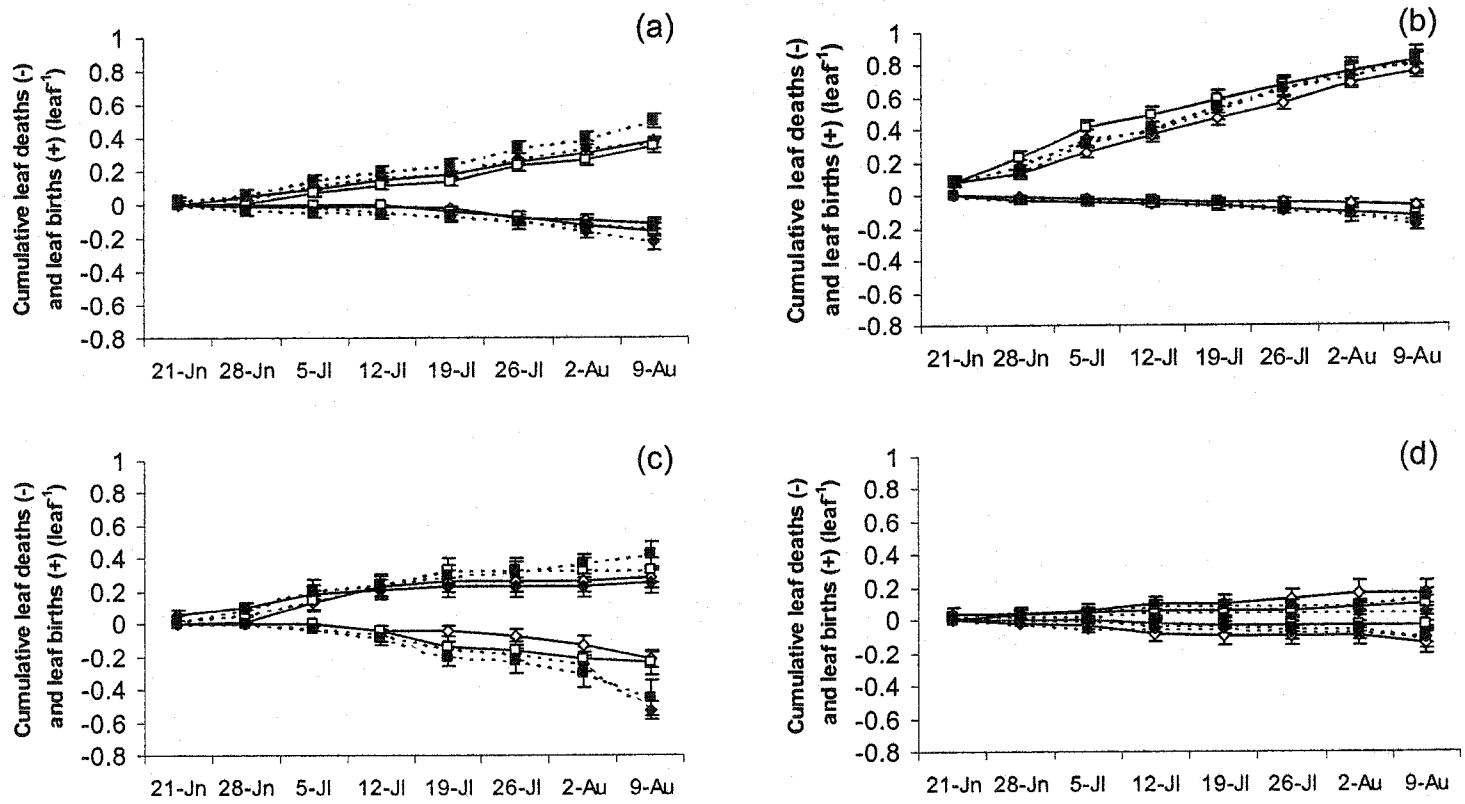


Fig. 2-1. Effect of treatment (control [◊], +N [♦], +T [□] and +T+N [■]) on cumulative leaf births and leaf deaths measured in 2001 for (a) *C. consimilis* (b) *D. octopetala*, (c) *P. bistorta* and (d) *S. reticulata*. Values are means ± 1SE.

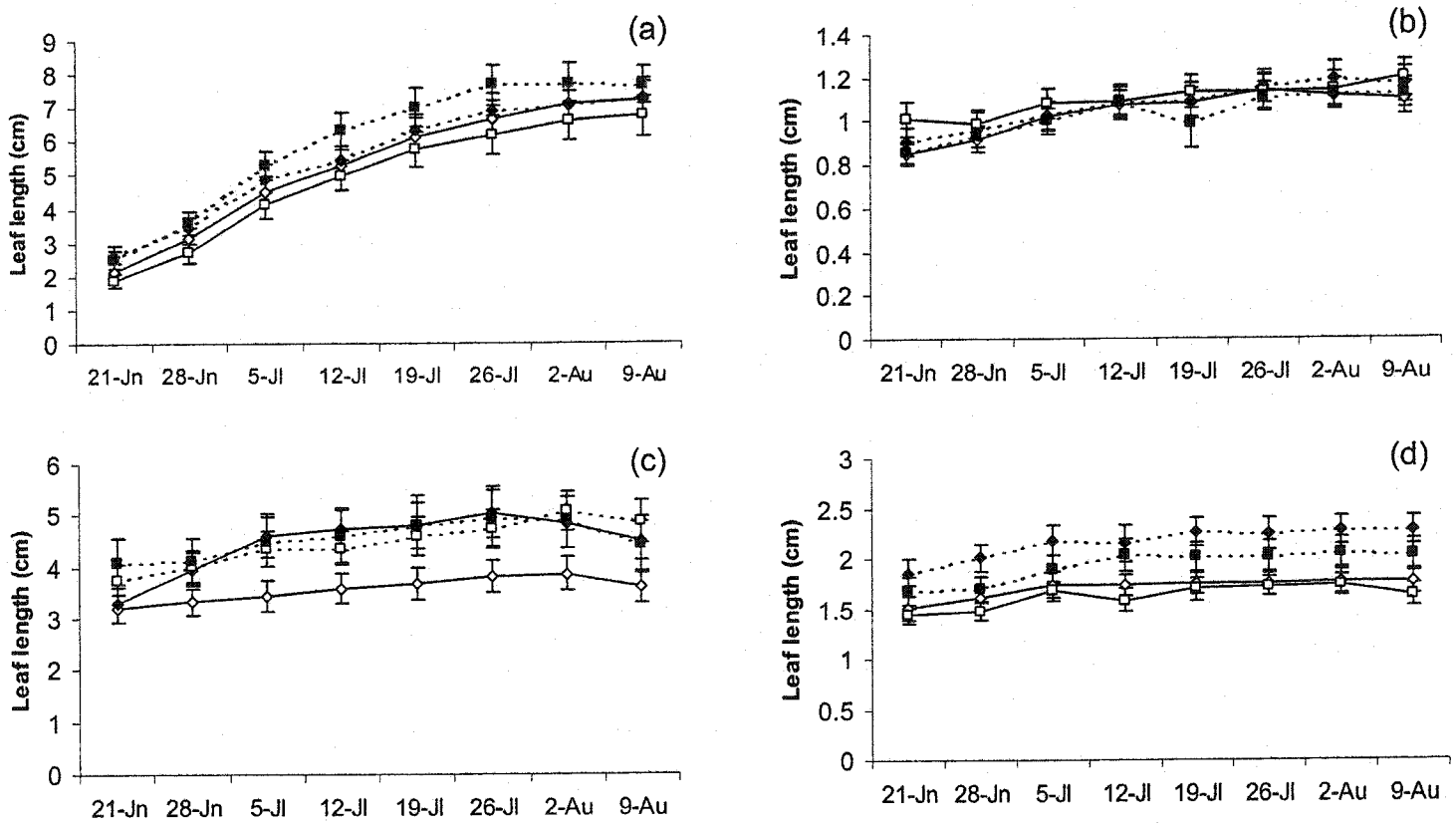


Fig. 2-2. Effect of treatment (control [◊], +N [♦], +T [□] and +T+N [■]) on leaf length measured in 2001 for (a) *C. consimilis,* (b) *D. octopetala*, (c) *P. bistorta* and (d) *S. reticulata*. Values are means ± 1SE.

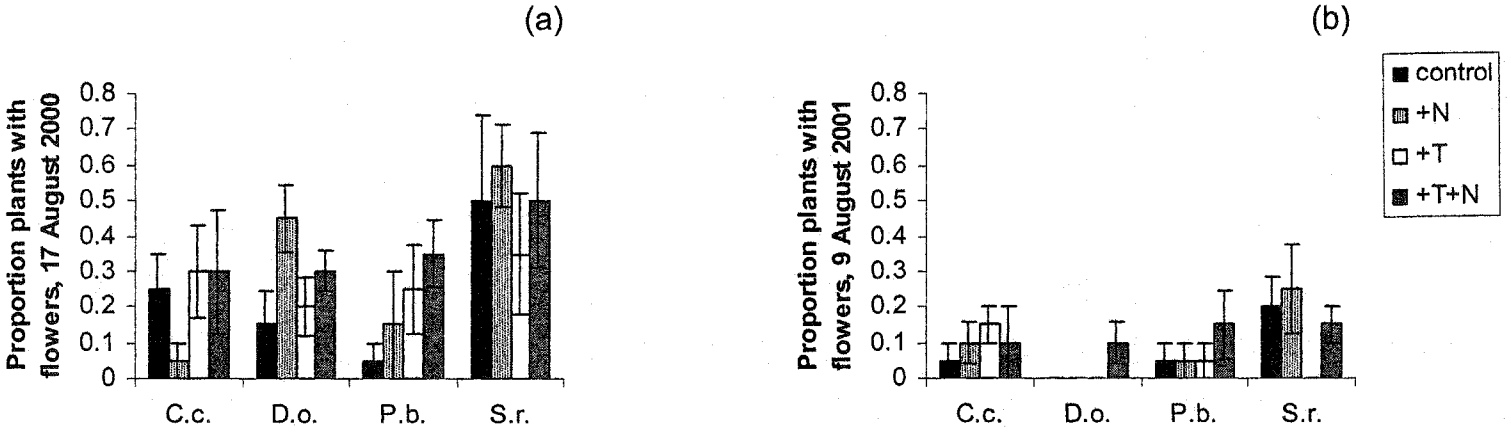


Fig. 2-3. Proportion of plants with flowers on (a) 17 August 2000 and (b) 9 August 2001 for *C. consimilis* (C.c.), *D. octopetala* (D.o.), *P. bistorta* (P.b.) and *S. reticulata* (S.r.). Values are means $\pm$ 1SE.

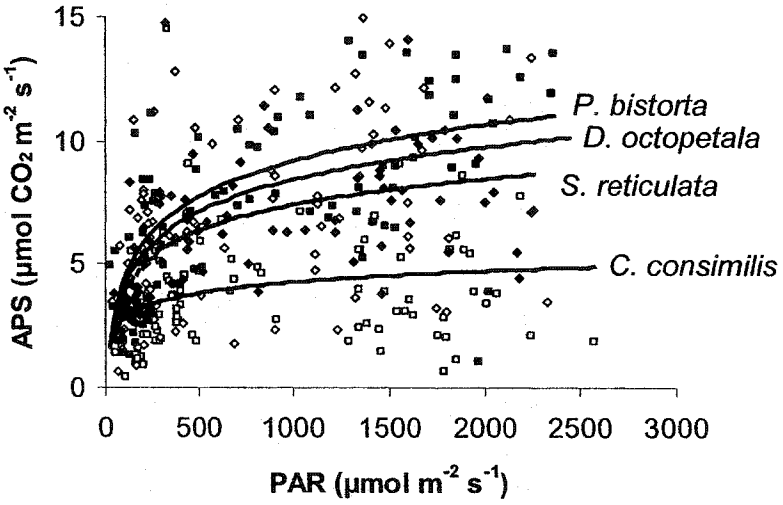


Fig. 2-4. Photosynthetic light response curves:
C. consimilis (□: APS=0.6597Ln(PAR)-0.2767, R^2=0.0804),
D. octopetala (◊: APS=1.88Ln(PAR)-4.564, R^2=0.2167),
P. bistorta (■: APS=2.0923Ln(PAR)-5.2338, R^2=0.3064) and
S. reticulata (♦: APS=1.5225Ln(PAR)–3.0993, R^2=0.3851) .

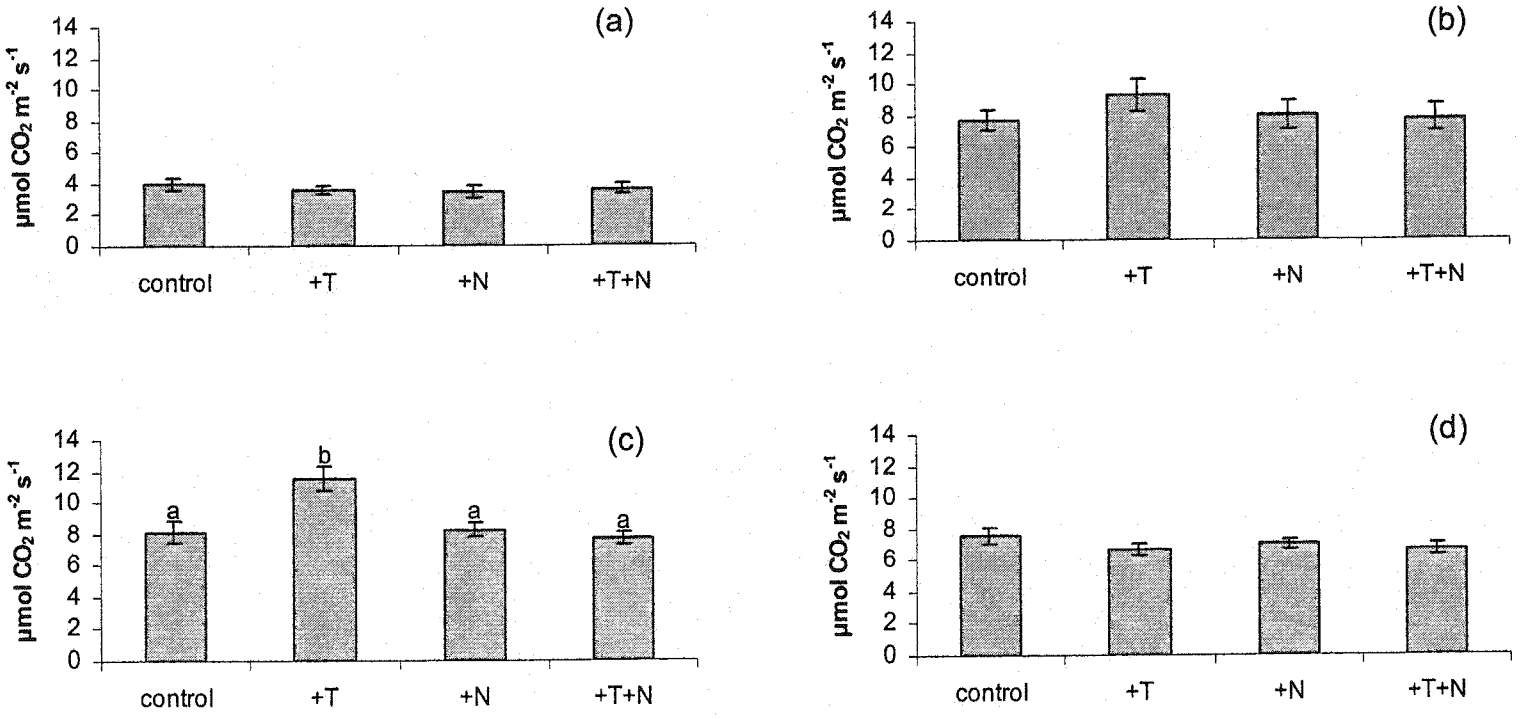
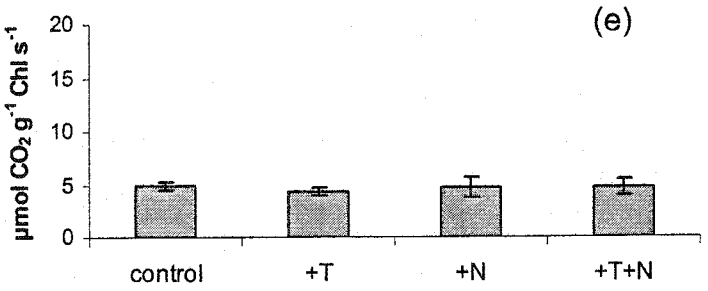
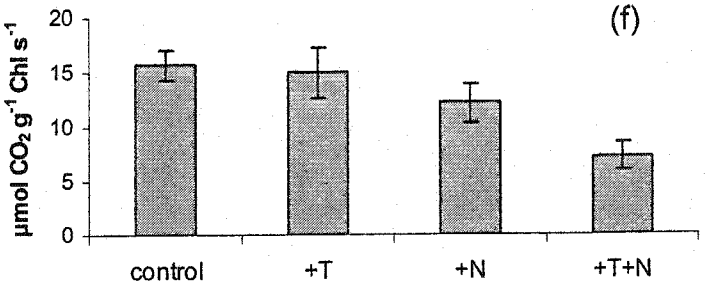
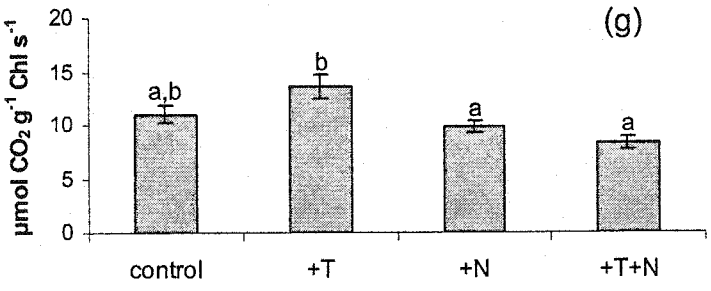
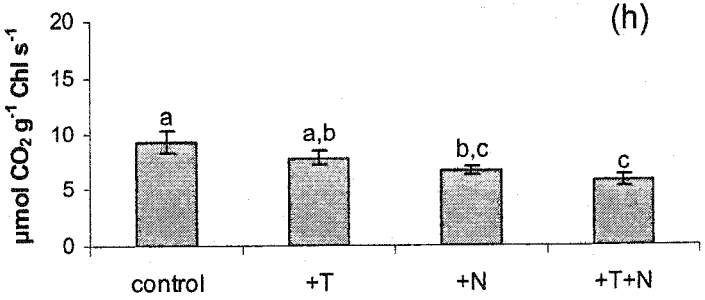


Figure 2-5. Rates of apparent photosynthesis on a leaf area basis (µmol CO_2 m^{-2} s^{-1}) and a chlorophyll basis (µmol CO_2 g^{-1} Chl s^{-1}) for (a, e) *C. consimilis*, (b, f) *D. octopetala*, (c, g) *P. bistorta* and (d, h) *S. reticulata* in control, warmed (+T), nitrogen addition (+N) and warmed and nitrogen addition (+T+N) plots. Letters indicate significant post-hoc differences (LSD, $P<0.05$, unadjusted). Values are means ± 1SE.


(e)
µmol CO2 g-1 Chl s-1
20
15
10
5
0
control
+T
+N
+T+N


(f)
µmol CO2 g-1 Chl s-1
20
15
10
5
0
control
+T
+N
+T+N


(g)
µmol CO2 g-1 Chl s-1
20
15
10
5
0
a,b
b
a
a
control
+T
+N
+T+N


(h)
µmol CO2 g-1 Chl s-1
20
15
10
5
0
a
a,b
b,c
c
control
+T
+N
+T+N

CHAPTER 3

Leaf demography, growth and photosynthetic capacity of two alpine plants in response to chronic and current season herbivory

Introduction

The response of individual plants to herbivory can be placed along a continuum from negative to positive (e.g. McNaughton et al. 1983, Maschinski and Whitham 1989, Hik and Jefferies 1990, Crawley 1997, Wegener and Odasz 1997, Paige 1999), depending upon conditions experienced at the time of defoliation. Influential biotic and abiotic factors include the intensity of defoliation, the amount of time for recovery, the growth rate of ungrazed plants, age of the grazed tissue, availability of nutrients and water, and grazing history (Nowak and Caldwell 1984, Maschinski and Whitham 1989, Oesterheld 1992, Crawley 1997).

In arctic and alpine environments the opportunities for compensation following herbivory are limited because plants are often found in areas with low nutrient and/or water availability, combined with the short period available for regrowth during the summer (Billings and Mooney 1968, Jefferies et al. 1994). However, several studies (Henry and Svoboda 1994, Jefferies et al. 1994, Diemer 1996, McIntire 1999, among others) suggest that under some conditions arctic and alpine plants are able to withstand carbon losses due to herbivory without negative consequences for plant vigor or reproduction. In many cases this facilitation is mediated by enhanced rates of nutrient cycling and nutrient redistribution leading to increased net aboveground production (Hik and Jefferies 1990, Henry and Svoboda 1994). Different growth forms utilize various

mechanisms for tolerating herbivory. For example, the large underground reserves of graminoids permit high loss of current year biomass, while evergreen shrubs and other slow growing species are more likely to protect photosynthetic tissues from herbivory with chemical defense compounds (Archer and Tieszen 1980).

The collared pika (*Ochotona collaris*) is a small lagomorph endemic to alpine areas of south and central Yukon and Alaska (MacDonald and Jones 1987). Collared pikas are generalist herbivores and due to perceived risk of predation, forage on plants directly adjacent to the boulderfields where they live. The intensity of foraging increases as the summer progresses as they collect food to be stored in hay piles that will be used during the winter. Since generations of pikas use the same boulderfields, plants growing within about three meters of the margin of the boulderfields are heavily grazed each year, while virtually no grazing occurs at a distance of over 6m (McIntire 1999). This natural grazing gradient allows for a comparison of grazing with few confounding effects, as outlined by McIntire and Hik (2002).

Several alpine species are able to withstand high grazing pressure at the end of the growing season (McIntire and Hik 2002). Mechanisms for dealing with tissue loss include higher rates of production of new leaves (*Kobresia myosuroides* and *Oxytropis nigrescens*) and a delay in leaf senescence (*Erigeron humilis*). We examined the responses of two common forage species, the sedge *Carex consimilis* Holm and the 'wintergreen' or semi-evergreen shrub *Dryas octopetala* L., to chronic (historical) and current year grazing in an alpine meadow in the southwest Yukon Territory, Canada. We measured plant growth by following leaf demography over the growing season and comparing photosynthetic rates and photosynthetic capacity during the mid to late

growing season. Plants close to talus (<2m) were considered historically grazed while those far from talus were considered historically ungrazed (>8m). Plants were considered ungrazed in the current growing season until herbivory was detected. We predicted that those plants with a history of grazing would compensate for herbivory through both demographic (increased leaf births, decreased leaf deaths or longer leaf lengths) and physiological (increased rates of photosynthesis) mechanisms. We also hypothesized that there would be a correlation between demographic and photosynthetic responses to effects of grazing.

Materials and Methods

Study Site

This study was conducted in the Ruby Range Mountains in southwestern Yukon Territory, Canada (61°13'N 138°16'W, elevation 1900m a.s.l.). *Dryas octopetala*, *Salix polaris* Wahlenb., *S. reticulata* L. and *Carex consimilis* are the dominant species, and boulderfields are interspersed throughout an essentially continuous alpine meadow (Price 1971, McIntire 1999). The snow-free growing season is less than 90 days and the site has low nutrient soils, characteristic of alpine sites.

Study Species

Carex consimilis is a loosely tufted, clonal sedge with rhizomatous growth (Cody 2000, Brooker et al 2001). It is part of the well-studied *Carex bigelowii* group, a Circumpolar Arctic-Montane species (Preston and Hill 1997). *C. consimilis* is found in a variety of habitats as it has a wide tolerance for varying climatic conditions, including

areas of late snowbeds and exposed ridge tops. *C. consimilis* usually reproduces vegetatively and consequently resources can be allocated among tillers, in part an adaptation to high levels of herbivory in many areas (Jónsdóttir and Callaghan 1989, Brooker et al 2001).

Dryas octopetala is a low, mat-forming shrub of the Rosaceae. It has a circumpolar distribution and is found in alpine regions of North America, Europe and Asia (Cody 2000). It dominates severe habitats with thin snow cover in winter and well-drained soil in summer. The leaves of *D. octopetala* are 'wintergreen', meaning that chlorophyll is broken down in the fall and the leaves become brown. These same leaves become photosynthetic the following spring and are usually functional for two years, after which they stay attached for another two to four years. 'Wintergreen' leaves allow for early season carbon gain, growth and reproduction (Welker et al. 1997). Unlike the graminoid *C. consimilis* which can continue to grow grazed leaves as the meristem usually escapes damage, *D. octopetala* must initiate growth of new leaves following herbivory. Extensive grazing of both *C. consimilis* and *D. octopetala* by *O. collaris* has been observed (Andruchow 2000).

Experimental Design

Five separate sites were chosen within a 4 ha area of the study site, based on the presence of a pika hay pile and observations of active pika grazing. At each location four 50cm X 50cm plots were established, two located 'close' to the margin of the boulderfield (<2m) and two located 'far' from the margin (>8m). These distances are based on previous observations of pika foraging behaviour (Huntly 1987, McIntire 1999).

One plot of each pair was left open to herbivory (control plots) and an herbivore exclosure consisting of 0.5 cm wire mesh was erected over the other (exclosure plots). This design provided four plots at each location: close-control, close-exclosure, far-control and far-exclosure. While we hoped to have ungrazed plants in exclosures and grazed plants in controls, presence or absence of grazing was monitored across the season for each plant. The number of grazed leaves was recorded for each plant on each sampling date. As pika grazing occurred across the season, sample sizes of ungrazed plants decreased across the growing season while those of grazed plants increased.

Leaf Demography

In summer 2001 five plants of *C. consimilis* and *D. octopetala* were marked with loops of coloured wire in each of the 20 plots. Leaf demography was monitored at weekly intervals on 1, 8, 15, 22, 29 July, and 5, 12 August. Number of leaves, number of recent leaf births, number of recent leaf deaths and leaf length were counted. New leaves were visible but not uncurled (*D. octopetala*) or less than 5mm in length (*C. consimilis*). Dead leaves were gently removed with scissors so that they would not be recounted at a later date. One leaf was randomly selected for length measurements and marked with a small dot of black ink. Because grazed leaves of *C. consimilis* remained functional it is possible that they were counted on more than one date. Grazed leaves of *D. octopetala* died soon after being grazed and therefore were not recounted. For this reason we reported the proportion of standing leaves grazed on each date for *C. consimilis* and the cumulative proportion of leaves grazed for *D. octopetala*.

Number of leaf births and deaths were standardized to the number of leaves on each plant at each date, and are reported 'per leaf' to minimize any effects of plant size and leaf number on growth. The cumulative number of leaf births and cumulative number of leaf deaths were calculated over the entire season, as well as rates of leaf births and leaf deaths per 7-day period.

Gas Exchange Measurements

Three plants of *C. consimilis* and *D. octopetala* were marked with a loop of coloured wire at each site for gas exchange and chlorophyll fluorescence measurements. The following combinations of distance and current year grazing were measured: close-grazed, close-ungrazed, and far-ungrazed. No far-grazed plants were measured because they were difficult to locate. To minimize the amount of handling, different plants from those used in the demography study were used.

All gas exchange measurements were made on 30 July 2001 using an Analytical Development Corporation (ADC) Model LCA-2 IRGA (Hoddeson, England) on fully expanded, green leaves. Due to the small size of the study species and difficulty in inserting only one leaf into the cuvette, a conifer cuvette (ADC model PLC 042A) was used and measurements were made on either whole plants or leaf clusters. Measurements were made on a day with no precipitation and full sunlight ($\geq$ 1800 μmol m^{-2} s^{-1}). For each measurement, once the gas exchange system had stablized, the plant was left in the cuvette for a minimum of 5 minutes. During this period, the difference in CO_2 (diff) value was recorded every 30 seconds, and temperature (temp), relative humidity (rh) and photosynthetically active radiation (PAR) were recorded every minute. Measured leaves

were traced onto paper in order to calculate leaf area. Apparent photosynthesis (APS) was then calculated using the formula:

$$APS = \text{air flow/leaf area}*(\text{diff } CO_2)*1.8$$

where air flow = 150 mL/min.

Rates on a μmol basis were determined by converting the volumetric flow rate to a mass flow rate using 1.8 μg/μL to determine the weight of CO_2 at NTP and 24.5 L/mole air at NTP (Hall et al 1993).

Equations were determined for each species to convert leaf areas to fresh mass and dry mass by measuring leaf area, fresh mass and dry mass for approximately ten leaves of each species at each of the five locations, both close and far from boulderfields. Linear regression was used to determine the relationship between leaf area and either fresh mass or dry mass. There were no significant differences in the relationship between leaf area and either fresh mass or dry mass based on distance, therefore the same equation was used for plants both close and far from boulderfields. Apparent photosynthesis was expressed as a rate per leaf area (μmol CO_2 m^{-2} s^{-1}), gram fresh mass (nmol CO_2 g^{-1}FM s^{-1}) and gram dry mass (nmol CO_2 g^{-1}DM s^{-1}).

Chlorophyll Fluorescence

All chlorophyll fluorescence measurements were made using a Hansatech Fluorescence Monitoring System (FMS 2, Hansatech, King's Lynn, UK) and fully expanded, green leaves. The steady state fluorescence under the ambient light regime, F_s, was measured. A saturating light pulse was then applied to measure the maximal fluorescence yield, F_m'. A 5-sec far-red pulse was then applied and the minimum yield,

F_o', recorded. The same area of leaf tissue used in the light-adapted measurements was then dark-adapted for 45 minutes using leaf clips. The minimal level of fluorescence, F_o, was measured by turning on the measuring light. Next, a saturating pulse of light was applied to measure the fluorescence maximum, F_m. The maximum quantum efficiency of PSII photochemistry was calculated as $F_v/F_m = (F_m - F_o)/F_m$, where F_v is variable fluorescence. Antennae efficiency of PSII [$F_v'/F_m' = (F_m'-F_o')/F_m'$], quantum efficiency of PSII [$\Phi_{PSII} = (F_m'-F_s)/F_m'$], photochemical quenching co-efficient [$qP = (F_m'-F_s)/(F_m'-F_o')$] and non-photochemical quenching co-efficient [$NPQ = (F_m-F_m')/F_m'$] were also calculated.

Data Analysis

A three-way repeated measures ANOVA ($\alpha = 0.05$) was used to test the main effects of distance (Dis), current season grazing (Gr) and date (DATE) on the demographic parameters measured for each species. The influence of the main effects on leaf length, cumulative leaf births and leaf deaths and rate of leaf births and deaths was tested for each species. Leaf length data was normal, however no transformation could normalize the leaf birth and leaf death data. However, because our sample sizes were quite large, the ANOVA analyses were robust to departures from normality (Sokal and Rohlf 1995; Underwood 1997). One consequence of a small number of cells with few samples in the ANOVA analyses is that formal means separation tests were not possible. Consequently, we present our results with standard error bars and emphasize the overall results of the ANOVA, rather than individual significant values.

A one-way ANOVA ($\alpha = 0.05$) was used to test the main effect of grazing and distance combinations (close-grazed, close-ungrazed, far-ungrazed) on gas exchange and fluorescence values for each species. Data met assumptions of normality and homogeneity of variance. The Least Significant Difference (LSD) test was used for post-hoc means separation tests (Carmer and Walker 1982).

To control for pseudoreplication, the random effect of plot was nested within the main effects (Underwood 1997). All statistical tests were conducted with SAS 8.1 statistical software (SAS Institute Inc. 1999).

Results

Baseline Demographic Patterns

During the 2001 growing season, 88% of *C. consimilis* and 73% of *D. octopetala* marked plants were grazed by 12 August in close-control plots (Fig. 3-1a,b). *C. consimilis* experienced the greatest intensity of grazing by 5 August with 48% of all standing leaves grazed in close-control plots (Fig. 3-1c). For *D. octopetala,* 39% of all leaves produced were grazed in close-control plots by 12 August (Fig. 3-1d). Grazing on plants in exclosures or far from the boulderfield were significantly lower. The maximum grazing intensity for *C. consimilis* was 4% (5 August), 8% (29 July) and 10% (12 August) for close-exclosures, far-controls, and far-exclosures, respectively (Fig. 3-1c). For *D. octopetala,* 16%, 13% and 11% of all leaves produced had been grazed by 12 August in close-exclosures, far-controls, and far-exclosures, respectively (Fig. 3-1d).

Irrespective of distance and current year grazing, cumulative leaf births were highest for *D. octopetala*, 0.46 ± 0.01, and lowest for *C. consimilis*, 0.26 ± 0.02, at the

end of the summer (Fig. 3-2). *C. consimilis* had the most leaf deaths, 0.08 $\pm$ 0.01 and *D. octopetala* had the fewest, 0.05 $\pm$ 0.01 (Fig. 3-2).

Effects of Current Year Grazing on Demography

Current season grazing had a significant effect on cumulative leaf deaths for *C. consimilis* (Table 3-1). Both far- and close- plants that were grazed in the current growing season had fewer leaf deaths than those that were not grazed (Fig. 3-3a). In contrast, cumulative leaf births were significantly affected by current year grazing for *D. octopetala* (Table 3-1). There was also a significant date X grazed interaction. By the end of the growing season (12 August) more leaf births were observed for plants that had been grazed in the current season compared to those that had not (Fig. 3-3b). The rate of leaf births was higher for grazed plants from 8 to 15 July than ungrazed plants.

Effects of Chronic (Historical) Grazing on Demography

Distance from boulderfield had a significant effect on leaf length for both species (Table 3-1). For *C. consimlis* there was a significant distance X date interaction. *C. consimilis* far-plants had longer leaves than close-plants by 29 July 2001, and this trend was evident until the end of the growing season (Fig. 3-4a). A two-way ANOVA showed no significant effect of either distance or grazing on leaf length at the beginning of the growing season (1 July; all P-values > 0.66). This suggests that the observed leaf length differences for close and far plants were the result of growth during the 2001 growing season. Far- *D. octopetala* plants had longer leaves than close- plants throughout the entire growing season (Fig. 3-4b). A significant effect of distance (two-way ANOVA, P

= 0.015) on *D. octopetala* leaf length was observed on 1 July suggesting that leaf length differences were not necessarily the result of current season growth as the difference was present from the onset of the measurements. There was also a significant current season grazing X distance interaction.

A significant effect of the 3-way interaction (current year X distance X date) was observed for *C. consimilis* cumulative leaf births as well as a significant date X distance interaction for rate of leaf births. On 15 July, far-grazed plants had the highest rate of leaf births. By the end of the growing season far-grazed and close-ungrazed plants had the highest levels of cumulative leaf births. Distance from boulderfield significantly affected rate of leaf deaths for *D. octopetala* and there was a significant date X distance interaction for cumulative leaf deaths. Close-plants, both grazed and ungrazed in the current growing season, had higher rates of leaf deaths than far-plants, particularly on 5 August when rates were highest, resulting in higher cumulative leaf deaths for close plants at the end of the growing season.

Gas Exchange Measurements

Irrespective of distance from boulderfield, *C. consimilis* plants had lower rates of APS, whether expressed on a leaf area, fresh mass or dry mass basis, compared with *D. octopetala* (Fig. 3-5). There was no significant effect of the combination of distance and current year grazing on *C. consimilis* rates of APS on any basis of expression (Table 3-2; Fig. 3-5). However, significant differences were observed for *D. octopetala* rates of APS (Table 3-2). Close-grazed plants, had significantly lower rates of APS than both close-ungrazed and far-ungrazed plants (Fig. 3-5). Differences were detected on a leaf area, fresh mass and dry mass basis.

Chlorophyll Fluorescence

Significant differences between the different combinations of distance and current season grazing were observed for the photochemical quenching co-efficient (qP) and non-photochemical quenching (NPQ) for *C. consimilis* (Table 3-2). Close-grazed plants had significantly lower values of qP and significantly higher values of NPQ (Fig 3-6b, c). No significant effect of any of the measured chlorophyll fluorescence parameters was observed for *D. octopetala* (Table 3-2; Fig 3-6).

Discussion

The number of grazed plants was highest in the close-control plots for both *C. consimilis* and *D. octopetala*. However, these values were not that much larger than the other plots, particularly for *C. consimilis*. While the exclosures kept out the larger vertebrate grazers (pikas, ground squirrels and marmots) they likely did not exclude microtine rodents and insects. Microtine grazing could account for the grazed plants observed in exclosure plots. However, the number of leaves grazed per plant was much higher in the close-controls for both species, compared to all other plots. The difference in number of leaves grazed between the different plots supports that those plants close to talus are grazed with the greatest intensity.

Leaf Births and Deaths

Differences in leaf births and deaths observed for the two species, irrespective of grazing and across all plots, were a reflection of different life forms and suggest different strategies for dealing with herbivory. *C. consimilis*, the sedge, had fewer leaf births than

the evergreen shrub *D. octopetala* by the end of the growing season. The basal meristem of *C. consimilis* allows for regrowth of grazed leaves after the grazing event (Kotanen and Jefferies 1989). *D. octopetala*, on the other hand, must replace grazed leaves with new ones as grazed leaves are no longer functional. Both species had low numbers of leaf deaths by the end of the growing season. Leaves of both plants can be functional for more than one growing season and therefore a slow turnover rate is not surprising (Archer and Tieszen 1980).

Our demographic results suggest that both historical and current year grazing are important influences on leaf births, deaths and lengths. The effect of current season grazing is more important than that reported by McIntire and Hik (2002) at the same site for three other grazed species. They found that only historical grazing was significant. A reduction in cumulative leaf deaths was observed for *C. consimilis* plants grazed in the current season. Other studies have also observed a delay in end of season senescence in response to herbivory (McNaughton 1979, Meyer 1998, McIntire and Hik 2002).

A significant effect of the interaction between current season grazing, distance from boulderfield and date was observed for cumulative leaf births of *C. consimilis*. Far-grazed plants showed a significant increase in cumulative leaf births on 15 July (Fig. 3-3a) and after this date had the highest number of cumulative leaf births of all the plots. Close-ungrazed plants showed a greater increase in cumulative leaf births than close-grazed plants on the last two dates, 5 August and 12 August. Grazing pressure was highest at this time (Fig. 3-1c). These differences suggest that low grazing pressure may stimulate leaf births while heavy grazing pressure may actually suppress leaf births.

D. octopetala plants grazed in the current season had more cumulative leaf births by the end of the growing season compared to those plants not grazed in the current season (Fig. 3-3b). On 8 July both close- and far- grazed plants had fewer cumulative leaf births than ungrazed plants. The following week (15 July), grazed plants had more cumulative leaf births than ungrazed plants. This is reflected in the high rates of leaf births for grazed plants during this time period. There was a substantial increase in the number of *D. octopetala* plants with grazing during this time period (Fig. 3-1b). The increase in cumulative leaf births indicated a response to grazing pressure.

The significant effect of distance from talus on *D. octopetala* rate of leaf deaths and the significant date by distance interaction on cumulative leaf deaths, suggest that close to talus plants have a higher turnover rate than plants far from talus. The increase in cumulative leaf births suggested that grazed plants have a higher turnover rate. This mechanism of dealing with herbivory has been observed in other studies (e.g. Archer and Tieszen 1980, Kotanen and Jefferies 1987).

Leaf Length

For both species, far-plants had longer leaves than close-plants (Fig. 3-4). Decreasing the amount of biomass per leaf while increasing the number of leaves on the plant is one mechanism of coping with herbivory and has been observed in grasses with a history of grazing (Fahnestock and Detling 1999). In this case, each leaf that was removed means less of a reduction in overall biomass and more available tissue for photosynthesis than if the plant had fewer, longer leaves. Most *D. octopetala* leaves senesced following a grazing event while those of *C. consimilis* continued to grow. This

was reflected in the decrease in leaf length of grazed *C. consimilis* plants on 5 August, while the same trend is not observed for *D. octopetala* (Fig. 3-4).

Gas Exchange and Chlorophyll Fluorescence

We were not able to examine the effects of current season grazing on far-plants, because all of the far-plants were ungrazed in the current season. We could have simulated herbivory by using a clipping treatment, however, such approaches can be problematic (Baldwin 1990, Paige 1999). Since we wished to look at the effects of pika grazing specifically, such a treatment would not be an appropriate method. Therefore, rather than separating current year and historical grazing we looked for differences between the three plots: close-grazed, close-ungrazed and far-ungrazed.

Consistent with the demography results, the gas exchange and chlorophyll fluorescence data suggest that *C. consimilis* and *D. octopetala* respond to grazing pressure in different ways. No significant effect of either historical or current year grazing was observed on gas exchange rates for *C. consimilis*. Nowak and Caldwell (1984) observed compensatory photosynthesis in two graminoid species following defoliation, however, differences between undefoliated and defoliated plants were observed only for specific foliage elements at specific times of development. They concluded that compensatory photosynthesis is not an important mechanism of herbivory tolerance for the two graminoid species. In a study of five grass species, Fahnestock and Detling (1999) failed to find any differences in photosynthetic rates of grazed and ungrazed plants in over 65% of their comparisons. Those cases where they did find

differences were for rates measured early in the growing season (May and June) and no differences were evident at the end of the growing season (August).

Even though measurements of APS showed no significant differences for *C. consimilis*, chlorophyll fluorescence measurements suggest that current season grazing had a significant effect on photosynthetic performance. Close-grazed plants had a lower value for proportion of open PSII (qP; Fig. 3-6b) and a higher value for non-photochemical quenching (NPQ; Fig. 3-6c). These results suggest that for grazed plants fewer PSII reaction centres are open, and as a consequence, more of the absorbed light energy is being used in non-photochemical processes (Maxwell and Johnson 2000). Therefore, grazing has the potential to decrease the amount of light energy being used in photosynthesis by *C. consimilis*.

D. octopetala plants close to boulderfields, grazed in the current growing season, had lower rates of APS than ungrazed plants in either the close or far plots (Fig. 3-5). The responses of *D. octopetala* to grazing provide no evidence of compensatory photosynthesis. Rather they suggest that herbivory has a negative effect on rates of APS. While several studies have shown compensatory photosynthesis following herbivory (e.g. Hodgkinson 1974, Painter and Detling 1981, Doescher 1997) this response is usually in the laboratory and/or after one defoliation event. Fewer studies have examined responses of APS to chronic herbivory, particularly in the field, and those that have suggest that compensatory photosynthesis is not an important element of herbivory tolerance (e.g. Nowak and Caldwell 1984).

The Relationship Between Demography and Physiological Measurements

We explicitly examined the effects of herbivory at more than one scale (i.e. physiological and morphological) and attempted to measure differences in photosynthesis and leaf demography across the growing season in grazed and ungrazed plants to look for any relationship between measurements at these two scales. A study by Fahnestock and Detling (1999) of grazed and ungrazed grasses found that the primary adaptations to grazing were more evident in morphological measurements rather than at physiological scales.

While we observed mechanisms for dealing with grazing in demographic measurements of *C. consimilis*, there was no evidence of increased photosynthesis to compensate for tissue lost to grazers. A study by Nowak and Caldwell (1984) attributed observed differences in gas exchange of grazed and ungrazed graminoids to delayed leaf senescense of defoliated plants. Our observation of fewer leaf deaths in grazed plants by the end of the growing season suggests a similar way of dealing with herbivory. The fewer number of leaf deaths by the end of the growing season suggests that the amount of photosynthetic tissue present on plants at the end of the growing season is higher than that on ungrazed plants. In this case, no difference in photosynthetic rates would be observed on a leaf area basis, however, more photosynthetic tissue on the grazed plants would result in greater end of season carbohydrate gain.

While it has been argued that belowground reserves are not an important factor in regrowth following defoliation (Richards 1986), this may not be the case for arctic-alpine plants which have a large proportion of their biomass belowground (Bliss 1971). Belowground biomass has been shown to play an important role in buffering the effects

of grazing for high Arctic sedges (Tolvanen and Henry 2000). A study of three graminoid species found that the species with the greatest belowground biomass had the most positive response to grazing while that with the least belowground biomass had the least competitive advantage. The underground reserves of *C. consimilis* could be buffering the loss due to grazing. Therefore, we would not expect to see an increase in photosynthetic rates as a response to herbivory. In addition, high photosynthetic rates at the end of the growing season (as described above) could be advantageous for replacing these depleted reserves.

An increase in leaf births was observed for grazed *D. octopetala* plants, however, this was not accompanied by an increase in photosynthetic rates. The opposite trend was observed with decreased rates of photosynthesis for grazed plants. Even though a negative response to grazing in terms of APS was observed for *D. octopetala*, its dominance, particularly close to talus where grazing pressure was high, suggests that it is able to cope with high levels of herbivory. One possible reason for not detecting compensatory photosynthesis was the timing of our measurements. In other studies, photosynthetic rates have been found to reach a peak shortly following the defoliation event, and then decline again (Hodgkinson 1974, Painter and Detling 1981, Parsons and Penning 1988). Perhaps the timing of our measurements missed such an increase. However, the herbivory by pikas at our site may not allow plants to recover, since they are constantly being grazed, particularly at the end of the growing season.

Studies that have observed compensatory photosynthesis usually do so following a complete release from herbivory, an unrealistic scenario for our site. Studies of photosynthetic rates across the growing season have found that they tend to decline as the

season progresses (Fahnestock and Detling 1999). Consequently, while differences in photosynthetic rates between defoliated and undefoliated plants were evident at the beginning of the growing season when rates were high, no difference was found at the end of the growing season. Those plants with a history of grazing may compensate with higher rates of photosynthesis at the beginning of the growing season, when grazing pressure is not as strong as it is at the end of the season. Further studies investigating photosynthetic rates across the entire growing season would be useful in determining if compensatory photosynthesis exists for the study species.

The increase in pika grazing intensity at the end of the growing season (McIntire 1999) is an interesting feature of this alpine community. The high grazing intensity, amount of time for recovery and low availability of water and nutrients suggest that the opportunities for recovery would be low. Additionally, some systems benefit from nutrient input from grazers (e.g. Hik and Jefferies 1990, Henry and Svoboda 1994), however, grazed plants at our site have no such benefit as pikas use latrines for urination and defecation (*O. princeps*: Aho et al. 1998). Nevertheless, it appears that *C. consimilis* and *D. octopetala* have developed mechanisms to withstand high levels of grazing pressure. These mechanisms were more apparent in demographic measurements than they were in measurements of photosynthesis and photosynthetic capacity.

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Table 3-1. Summary of F-values from RM-ANOVAs for leaf demographic characters. Main effects are Distance (Dis), Grazed (Gr) and sample period throughout the summer (Date). Significant values are indicated in bold.

C. consimilis

	Cumulative leaf births		Cumulative leaf deaths		Rate of leaf births		Rate of leaf deaths		Leaf length	
	$F_{(numdf, dendf)}$	P	$F_{(numdf, dendf)}$	P	$F_{(numdf, dendf)}$	P	$F_{(numdf, dendf)}$	P	$F_{(numdf, dendf)}$	P
Distance	$0.21_{(1,35)}$	0.6510	$0.22_{(1,35)}$	0.6451	$0.84_{(1,35)}$	0.3658	$0.02_{(1,35)}$	0.8991	**$4.54_{(1,35)}$**	**0.0402**
Grazed	$0.64_{(1,35)}$	0.4278	**$6.83_{(1,35)}$**	**0.0131**	$0.01_{(1,35)}$	0.9415	$0.21_{(1,35)}$	0.6508	$0.36_{(1,35)}$	0.5504
Dis X Gr	**$7.93_{(1,35)}$**	**0.0079**	$0.11_{(1,35)}$	0.7371	$0.90_{(1,35)}$	0.3483	$0.00_{(1,35)}$	0.9853	$0.83_{(1,35)}$	0.3699
Date	**$98.71_{(6,636)}$**	**<0001**	**$29.95_{(6,636)}$**	**<0001**	$0.64_{(6,629)}$	0.7007	**$7.28_{(6,629)}$**	**<0001**	**$85.44_{(6,617)}$**	**<0001**
Date X Dis	**$3.95_{(6,636)}$**	**0.0007**	$0.43_{(6,636)}$	0.8571	**$2.31_{(6,629)}$**	**0.0328**	$0.72_{(6,629)}$	0.6376	**$10.12_{(6,617)}$**	**<0001**
Date X Gr	$1.36_{(6,636)}$	0.2304	$0.99_{(6,636)}$	0.4312	$1.51_{(6,629)}$	0.1708	$0.27_{(6,629)}$	0.9495	$1.00_{(6,617)}$	0.4219
Date X Dis X Gr	**$4.66_{(6,636)}$**	**0.0001**	$0.77_{(6,636)}$	0.5954	$1.22_{(6,629)}$	0.2955	$0.83_{(6,629)}$	0.5450	$0.15_{(6,617)}$	0.9890

D. octopetala

	Cumulative leaf births		Cumulative leaf deaths		Rate of leaf births		Rate of leaf deaths		Leaf length	
	$F_{(numdf, dendf)}$	P	$F_{(numdf, dendf)}$	P	$F_{(numdf, dendf)}$	P	$F_{(numdf, dendf)}$	P	$F_{(numdf, dendf)}$	P
Distance	$0.73_{(1,36)}$	0.4000	$3.40_{(1,36)}$	0.0735	$0.54_{(1,36)}$	0.4670	**$4.74_{(1,36)}$**	**0.0361**	**$28.56_{(1,36)}$**	**<0001**
Grazed	**$10.87_{(1,36)}$**	**0.0022**	$0.03_{(1,36)}$	0.8739	$0.64_{(1,36)}$	0.4296	$0.01_{(1,36)}$	0.9142	$1.24_{(1,36)}$	0.2722
Dis X Gr	$0.04_{(1,36)}$	0.8383	$0.47_{(1,36)}$	0.4991	$0.36_{(1,36)}$	0.5540	$1.09_{(1,36)}$	0.3027	**$4.35_{(1,36)}$**	**0.0442**
Date	**$135.53_{(6,634)}$**	**<0001**	**$14.59_{(6,634)}$**	**<0001**	**$3.32_{(6,632)}$**	**0.0031**	**$3.10_{(6,632)}$**	**0.0054**	**$5.25_{(6,626)}$**	**<0001**
Date X Dis	$1.71_{(6,634)}$	0.1155	**$4.68_{(6,634)}$**	**0.0001**	$1.82_{(6,632)}$	0.0936	$2.00_{(6,632)}$	0.0634	$0.62_{(6,626)}$	0.7176
Date X Gr	**$2.50_{(6,634)}$**	**0.0213**	$0.21_{(6,634)}$	0.9728	**$2.95_{(6,632)}$**	**0.0076**	$1.21_{(6,632)}$	0.2997	$1.80_{(6,626)}$	0.0970
Date X Dis X Gr	$0.25_{(5,634)}$	0.9420	$0.44_{(5,634)}$	0.8197	$0.05_{(5,632)}$	0.9986	$0.54_{(5,632)}$	0.7468	$1.48_{(5,626)}$	0.1933

Table 3-2. Summary of F-values from one-way ANOVAs for apparent photosynthesis (APS) and chlorophyll fluorescence parameters. The independent effect was plot (close-grazed, close-ungrazed and far-ungrazed). Numerator df = 2 and denominator df = 17. Significant values are indicated in bold.

	C. consimilis		*D. octopetala*	
	F	P	F	P
APS (μmol CO_2 m^{-2} s^{-1})	0.02	0.9832	**6.92**	**0.0063**
APS (nmol CO_2 g^{-1}FM s^{-1})	0.02	0.9781	**7.77**	**0.0040**
APS (nmol CO_2 g^{-1}DM s^{-1})	0.02	0.9786	**8.03**	**0.0035**
F_v/F_m	1.02	0.3831	2.58	0.1047
F_v'/F_m'	0.13	0.8763	0.08	0.9210
Φ_{PSII}	3.11	0.0709	0.12	0.8844
qP	**3.68**	**0.0483**	0.10	0.9077
NPQ	**7.29**	**0.0052**	0.18	0.8343

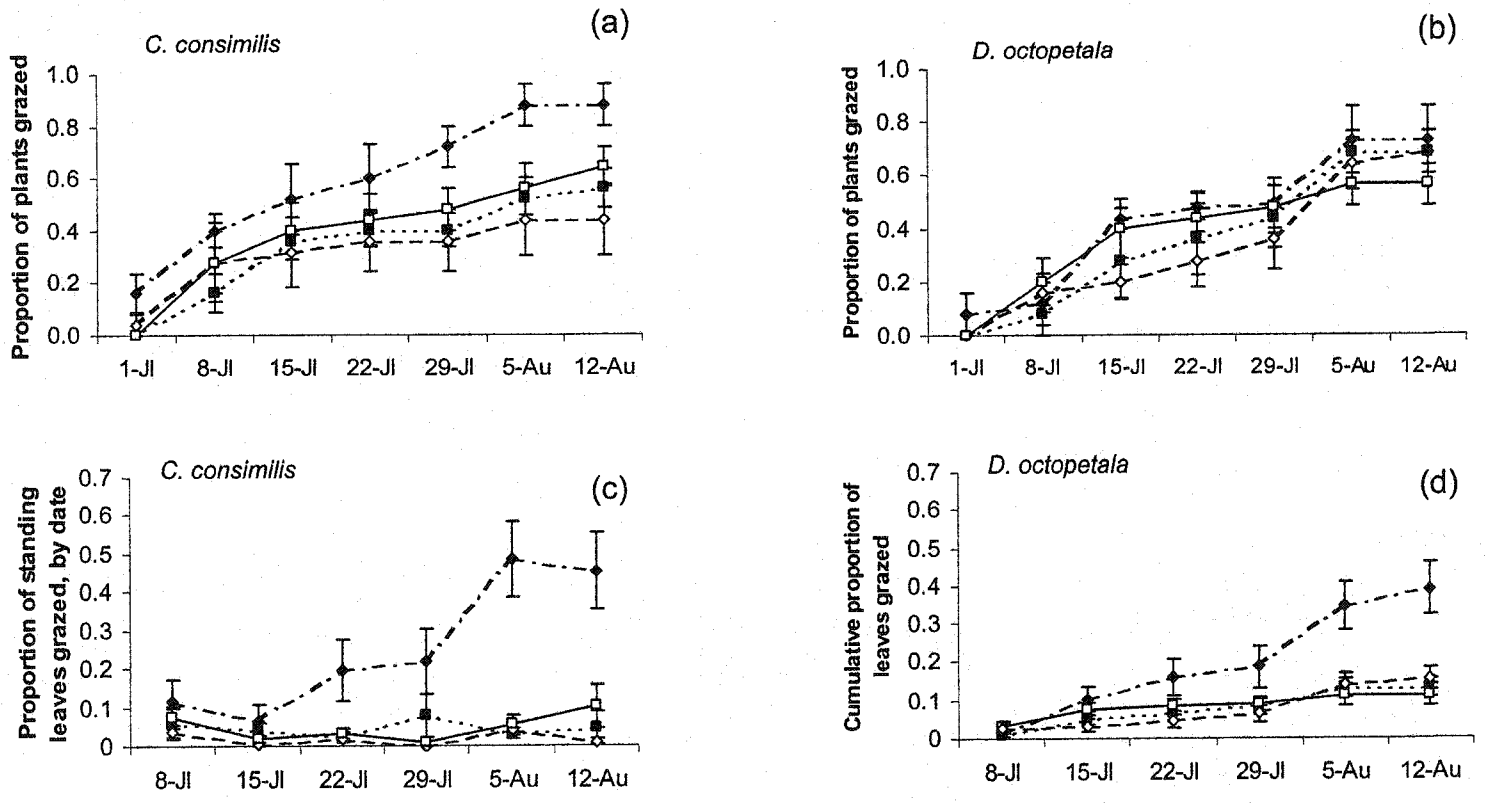

Fig. 3-1. Grazing in plots, by date. (a) and (b) occurrence, measured as the proportion of plants with detected grazing. (c) and (d) intensity, measured as proportion of leaves grazed per plant. Values are means ± 1SE.
Symbols: ♦ close-control, ◊ close-exclosure, ■ far-control and □ far- exclosure.

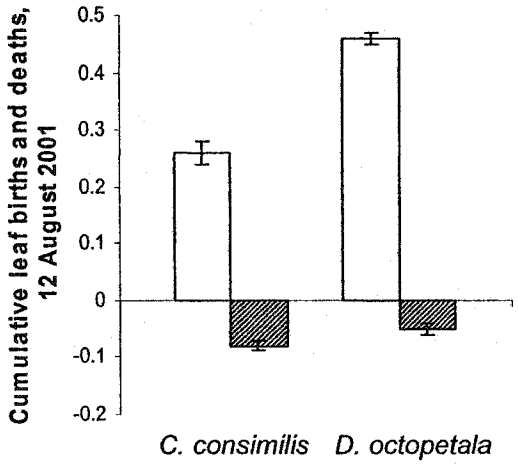

Fig. 3-2. Total cumulative leaf births (+) and leaf deaths (-), per leaf, on 12 August 2001. Values are means $\pm$ 1SE .

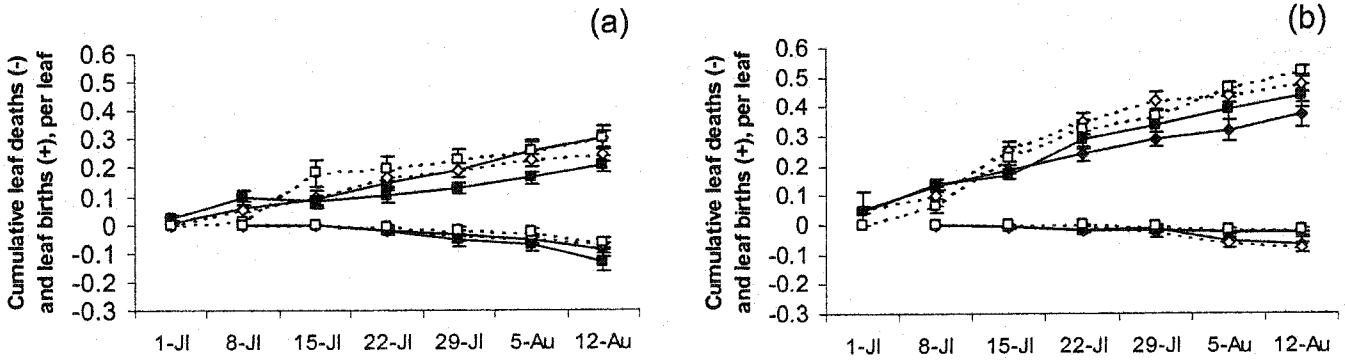

Fig. 3-3. Effect of distance from boulderfield (□ far, ♦ close) and current year grazing (solid - not grazed, open - grazed) on cumulative leaf births and deaths for both species, by date (2001). (a) *C. consimilis* and (b) *D. octopetala*. Values are means ± 1SE.

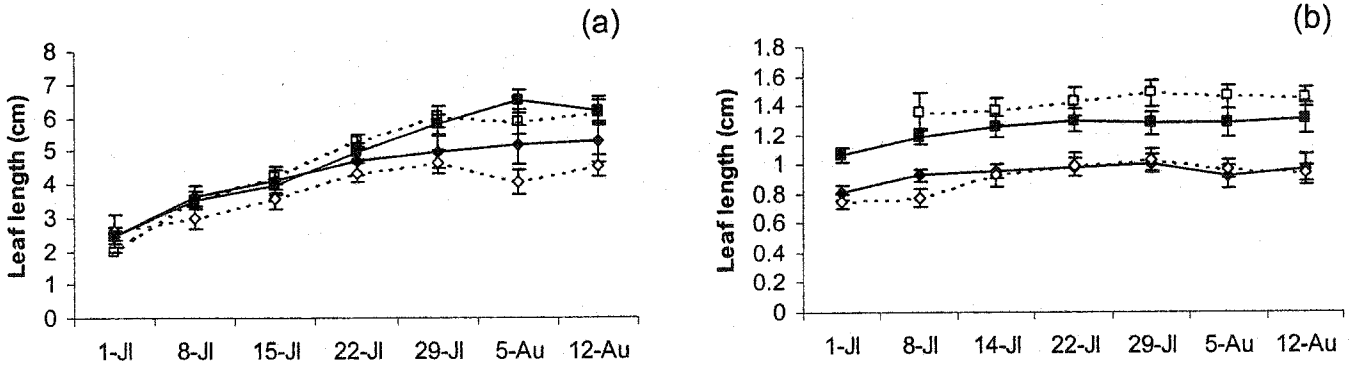

Fig. 3-4. Effect of distance from boulderfield (□ far, ♦ close) and current year grazing (solid - not grazed, open - grazed) on leaf length for both species, by date (2001). (a) *C. consimilis* and (b) *D. octopetala.*
Values are means ± 1SE.

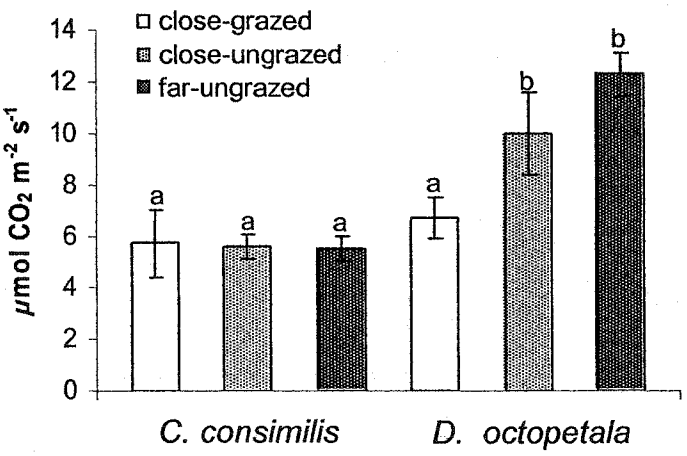

Fig. 3-5. Apparent photosynthesis (APS) for *C.* consimilis and *D. octopetala* plants with different combinations of distance and current season grazing. Values are means ± 1SE, expressed on a leaf area basis. Letters indicate significant post-hoc differences (LSD, $P<0.05$, unadjusted).

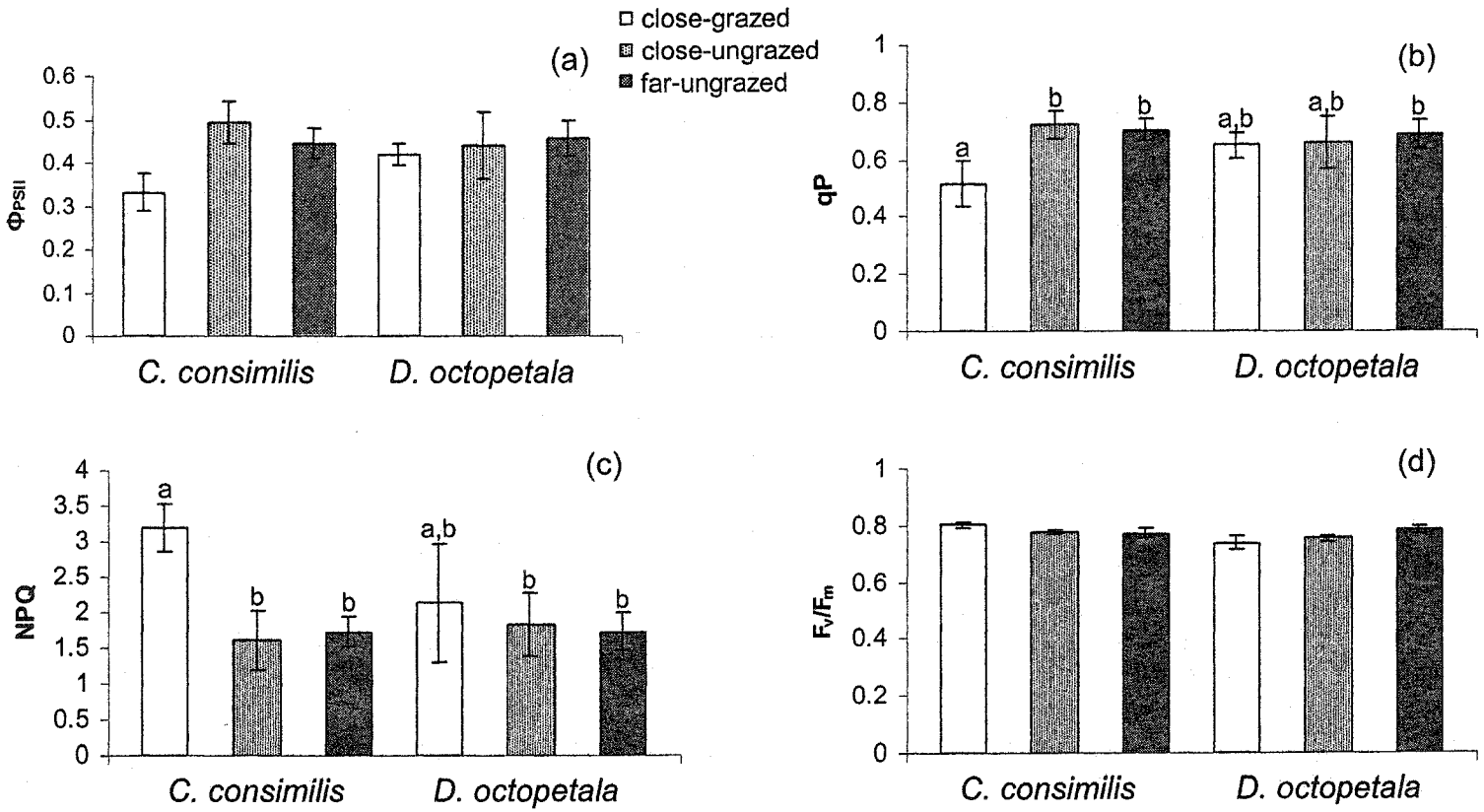

Fig. 3-6. Chlorophyll fluorescence measures for *C. consimilis* and *D. octopetala* with different combinations of distance and current season grazing. (a) Quantum efficiency of photosystem II, (b) portion of open PSII, (c) non-photochemical quenching and (d) maximum quantum yield of photosystem II from dark-adapted plants. Values are means ± 1SE and letters indicate significant post-hoc differences (LSD, $P<0.05$, unadjusted).

CHAPTER 4

An assessment of the relationship between photosynthesis, growth form and functional grouping of alpine plants in the southwest Yukon

Introduction

Predictions about how ecosystems will respond to environmental change require an understanding of the ecophysiology of the species present. While detailed information for all species in a community is difficult to obtain, many studies have overcome this problem is by categorizing plants into functional groups based on common structural and/or process features (Körner 1994, Chapin et al. 1996, Díaz and Cabido 1997). The difference between functional groups and more traditional taxonomic systems is that rather than forming groups based on phylogeny, species are grouped based on resource use and response to biotic and environmental controls (Duckworth et al. 2000). In arctic-alpine communities, vascular plants species are usually classified as being either herbaceous perennial, deciduous dwarf shrub, evergreen dwarf shrub or graminoid. (e.g. Webber 1978, Walker et al. 1989, Chapin et al. 1996).

Functional groups are particularly useful for summarizing individual species responses into general recurrent patterns and to model responses from the single-leaf level to a larger scale (Körner 1994, Díaz et al. 1998, Arft et al. 1999). In the Arctic, some studies have predicted and found high similarity of response within growth forms (e.g. Chapin et al. 1996, Arft et al. 1999), but there is also considerable variation in physiology and growth of closely related species.

Chapin et al. (1996) used cluster analysis to show that using arctic plant growth forms as functional groups in regional models predicting response to climate change was acceptable. Their analysis was performed using traits expected to influence ecosystem processes and the resulting grouping was similar to the traditional growth forms used in arctic botany. In contrast, Oberbauer and Oechel (1989) examined the natural grouping of arctic species based on photosynthetic rates to see if they coincided with growth form and found only partial correspondence between the two measures. In a meta-analysis of field experiments simulating climate change, Dormann and Woodin (2002) found that generalizing responses by plant functional groups provided little distinction between groups.

An adequate understanding and description of functional groups is essential for constructing and refining global and regional scale models of vegetation change (Smith et al. 1993). Many different plant growth-form criteria have been applied to describe vegetation (e.g. Warming 1909, Raunkiaer 1934, Whittaker 1975, Box 1981), but further sophistication and validation is necessary as the application of the functional group concept becomes widely adopted. Epstein et al. (2001) found that the level of species aggregation greatly affected the outcome of a regional-scale model. The study suggests that over-simplifying patterns too much could result in incorrect predictions (as discussed by Walker 2000).

This study examined variation in apparent photosynthesis (APS) and related traits (chlorophyll fluorescence and stomatal density) for 10 alpine vascular plant species, representing the four common growth forms. These traits are important in resource acquisition and have been used in other species groupings (e.g. Chapin et al. 1996). We

predicted that species representing the same growth form would have similar rates of APS. We also predicted that the lowest rates of APS would be those of the evergreen shrubs while the graminoids, deciduous shrubs and perennial forbs would have similar rates of APS, based on duration of photosynthetic activity for each growth form throughout the growing season. In addition to the rates we measured, we also summarized photosynthetic rates of arctic-alpine species reported in the literature to further investigate the relationship between photosynthesis, growth form and classification of functional groups of arctic-alpine species.

Materials and Methods

Study Site and Study Species

This study was conducted in the Ruby Range Mountains in southwestern Yukon Territory, Canada (61°13'N 138°16'W, elevation 1900m a.s.l.). The site consists of boulderfields interspersed throughout an essentially continuous alpine meadow (Price 1971, McIntire 1999). Over one hundred alpine tundra plant species have been identified in the region, with *Dryas octopetala* L., *Salix polaris* Wahlenb., *S. reticulata* L. and *Carex consimilis* Holm being the dominant species (McIntire 1999). The snow-free growing season is less than 90 days and the site has low nutrient soils, characteristic of alpine sites. This study focused on 10 species from four functional groups, selected on the basis of growth form (Table 4-1).

Five transects approximately 30 m apart were established in mid-July 2001, each running parallel to a shallow creek bed. Along each transect one plant of each species

was selected for gas exchange and chlorophyll fluorescence measurements. Plants were surveyed in a random order and were at least 2m apart.

Gas Exchange Measurements

Gas exchange measurements were made using an Analytical Development Corporation (ADC) Model LCA-2 IRGA (Hoddeson, England) on fully expanded, green leaves. Due to the small size of the study species and difficulty in inserting only one leaf into the cuvette, a conifer cuvette (ADC model PLC 042A) was used and measurements were made on either whole plants or leaf clusters of fully expanded green leaves. Measurements were made between 20 to 22 July 2001 on days with no precipitation and full sunlight ($\geq$ 1800 µmol m^{-2} s^{-1}). For each measurement, once the gas exchange system had stablized, the plant was left in the cuvette for a minimum of 5 minutes. During this period, the difference in CO_2 (diff) was recorded every 30 seconds, and temperature (temp), relative humidity (rh) and photosynthetically active radiation (PAR) were recorded every minute. Measured leaves were traced onto paper in order to calculate leaf area. Apparent photosynthesis (APS) was then calculated using the formula:

$$APS = \text{air flow/leaf area} * (\text{diff } CO_2) * 1.8$$

where air flow = 150 mL/min.

Rates on a µmol basis were determined by converting the volumetric flow rate to a mass flow rate using 1.8 µg/µL to determine the weight of CO_2 at NTP and 24.5 L/mole air at NTP (Hall et al. 1993).

Equations were determined for each species to convert leaf areas to fresh mass and dry mass by measuring leaf area, fresh mass and dry mass for approximately ten

leaves of each species at each of the five locations. Linear regression was used to determine the relationship between leaf area and either fresh mass or dry mass. Apparent photosynthesis was expressed as a rate per leaf area (μmol CO_2 m^{-2} s^{-1}), per gram fresh mass (nmol CO_2 g^{-1}FM s^{-1}) and per gram dry mass (nmol CO_2 g^{-1} DM s^{-1}).

Chlorophyll Fluorescence

Fluorescence measurements were made using a Hansatech Fluorescence Monitoring System (FMS 2, Hansatech, Kings Lion, UK) on fully expanded, green leaves. Steady state fluorescence under the ambient light regime, F_s, was measured, and then a saturating light pulse was applied to measure the maximal fluorescence yield, F_m'. A 5 s far-red pulse was then applied to determine the minimum yield, F_o'. The same area of leaf tissue used in the light adapted measurements was then dark-adapted for 45 min using leaf clips. The minimal level of fluorescence, F_o, was measured by turning on the measuring light. Next, a saturating pulse of light was applied to measure the fluorescence maximum, F_m. The maximum quantum efficiency of PSII photochemistry was calculated as $F_v/F_m = (F_m - F_o)/F_m$, where F_v is variable fluorescence. Antennae efficiency of PSII [$F_v'/F_m' = (F_m'-F_o')/F_m'$], quantum efficiency of PSII [$\Phi_{PSII} = (F_m'-F_s)/F_m'$], photochemical quenching co-efficient [$qP = (F_m'-F_s)/(F_m'-F_o')$] and non-photochemical quenching co-efficient [$NPQ = (F_m-F_m')/(F_m-F_o')$] were also calculated.

Stomatal Density

Stomatal density was determined by painting a representative leaf with clear nail polish, allowing it to dry and then peeling it from the leaf surface. The resulting impression was then examined under a light microscope. Four counts of distinct areas

were made per sample, avoiding 1) veins and 2) leaf edges, each of an area of approximately 1mm^2. Leaves were chosen using the same criteria as those chosen for gas exchange and fluorescence measurements. Due to the nature of the leaf surface, suitable impressions were not obtained for *Cassiope tetragona* and *Dryas octopetala*.

Literature Survey

Rates of APS were compiled from the following studies: Forbs: Tieszen and Johnson (1975), Körner and Diemer (1987), Oberbauer and Oechel (1989), Körner and Diemer (1994), Wookey et al. (1994), Bowman et al. (1995), Loik et al. (2000), Starr et al. (2000). Deciduous Shrubs: Tieszen (1975), Oberbauer and Oechel (1989), Bowman and Conant (1994). Evergreen Shrubs: McGraw (1987), Oberbauer and Oechel (1989), Wookey et al. (1995), Welker et al. (1997). Graminoids: Tieszen (1973), Tieszen (1975), Tieszen and Johnson (1975), Körner and Diemer (1987), Oberbauer and Oechel (1989), Bowman et al. (1995). Only studies that reported rates as Γmol CO_2 m^{-2} s^{-1} were used. There was considerable variation in instrumentation and methodology that we did not attempt to account for.

Data Analysis

Gas exchange rates, fluorescence values and stomatal density were analyzed using a one-way ANOVA ($\alpha = 0.05$) with growth form as the main effect to test for functional group differences. To test for species differences, a one-way ANOVA ($\alpha = 0.05$) with species as the main effect was used. Data met assumptions of normality and homogeneity of variance. The Least Significant Difference (LSD) method was used for

post-hoc comparisons of factors found to be significant in the ANOVAs. All statistical analyses were conducted using the statistical software package SAS 8.1 (SAS Institute Inc. 1999).

Results

Gas Exchange

Significant differences in rates of APS were observed at both the species and growth form levels (Table 4-2). The deciduous shrub *S. polaris* had the highest rate of APS on a leaf area basis (11.71 $\pm$ 1.60 $\mu molCO_2\ m^{-2}\ s^{-1}$) and the evergreen shrub *C. tetragona* had the lowest (2.65 $\pm$ 0.34 $\mu molCO_2\ m^{-2}\ s^{-1}$) (Fig. 4-1). In terms of growth form, perennial forbs and deciduous shrubs had the highest rates of APS, at 9.61 $\pm$ 0.89 $\mu molCO_2\ m^{-2}\ s^{-1}$ and 9.53 $\pm$ 0.79 $\mu molCO_2\ m^{-2}\ s^{-1}$, respectively. Evergreen shrubs were intermediate (7.08 $\pm$ 1.71 $\mu molCO_2\ m^{-2}\ s^{-1}$). Graminoids had the lowest rate at 5.66 $\pm$ 0.82 $\mu molCO_2\ m^{-2}\ s^{-1}$, significantly lower than both the forbs and deciduous shrubs. Generally, species of the same growth form had similar rates of APS. The one exception was the evergreen shrubs, where *D. octopetala* (11.51 $\pm$ 1.78 $\mu molCO_2\ m^{-2}\ s^{-1}$) was significantly higher than *C. tetragona* (2.65 $\pm$ 0.34 $\mu molCO_2\ m^{-2}\ s^{-1}$).

Chlorophyll Fluorescence

There were no significant differences for any of the measured fluorescence parameters (Table 4-2). A range of values between 0.724 $\pm$ 0.324 and 0.816 $\pm$ 0.365 were obtained for F_v/F_m, 0.527 $\pm$ 0.034 and 0.699 $\pm$ 0.067 for F_v'/F_m', 0.317 $\pm$ 0.033 and

0.603 ± 0.048 for Φ_{PSII}, 0.501 ± 0.047 and 0.868 ± 0.035 for QP, and 1.393 ± 0.414 and 3.137 ± 0.317 for NPQ.

Stomatal Density

There were significant differences between species for stomatal density (Table 4-2). *S. reticulata* had the highest stomatal density, 47.9 ± 2.5 mm^{-2}, and *P. frigidus* had the lowest density, 15.4 ± 0.8 mm^{-2} (Table 4-3). Three species, *S. polaris*, *E. latifolium* and *P. bistorta* were found to be amphistomatous. *E. latifolium* had a higher density on its adaxial surface than its abaxial surface, while the opposite was observed for *S. polaris* and *P. bistorta*. All other species were hypostomatous.

There were also significant differences between the three growth forms in terms of stomatal density (Table 4-2). Deciduous shrubs had a significantly higher density, 34.1 ± 2.4 mm^{-2}, than perennial forbs, 19.2 ± 1.0 mm^{-2}. Graminoids, had an intermediate density, 25.0 ± 1.9 mm^{-2}, neither significantly lower than deciduous shrubs nor higher than perennial forbs.

Comparison with Literature Values

Photosynthetic rates for forty-nine arctic-alpine plant species were compiled from fourteen different studies. At the species level both the highest and lowest APS rates observed in the literature were for perennial forbs (Fig. 4-2): *Saxifraga cernua* had the lowest rate (4.1 µmol CO_2 m^{-2} s^{-1}) and *Ligusticum mutellina* had the highest (23.9 µmol CO_2 m^{-2} s^{-1}) (Tieszen and Johnson 1975, Körner and Diemer 1987). Significant differences were observed between the four different growth forms in the literature

survey (P = 0.029). Perennial forbs had the highest rates of APS and evergreen shrubs had the lowest, with values of 15.0 ± 5.0 μmol CO_2 m^{-2} s^{-1} and 11.2 ± 4.4 μmol CO_2 m^{-2} s^{-1}, respectively. The APS rates of perennial forbs were significantly higher than those of graminoids and deciduous shrubs. No significant differences were observed between evergreen shrubs and any other growth form.

Discussion

We included only ten locally dominant plant species in our study of plant functional groups. It has been hypothesized that species of the same growth form will respond similarly to environmental changes because of similarities in inherent physiological pathways (Arft et al. 1999). Understanding if species of the same growth form are similar to one another would allow for general predictions to be made based on detailed studies of a few representatives of each group. For example, the results of our studies of warming and nitrogen addition (Chapter 2) and herbivory (Chapter 3) could be extrapolated to other species of the same growth form.

Gas Exchange

As predicted, perennial forbs and deciduous shrubs had the highest rates of APS on a leaf area basis. While both rates were higher than for evergreen shrubs, these differences were not significant. Contrary to our hypothesis, the two graminoid species had the lowest rate of APS in our study. The large difference in APS between the two evergreen species increased the variance, and resulted in rates not significantly lower than the perennial forbs and deciduous shrubs and not significantly higher than the graminoids (Fig. 4-1).

C. tetragona had the lowest rate (2.65 ± 0.34 ΠmolCO$_2$ m^{-2} s^{-1}), significantly lower than all of the study species except for the two graminoids, *C. consimilis* and *F. altaica*. Similarly, in an arctic study of the CO_2-assimilation rates of nineteen representatives of the four tundra growth forms, the rate of *C. tetragona* was the lowest of all the studied species. Different from our growth form results, evergreens were found to have the lowest rates. However, *D. octopetala* was not included in the arctic study (Oberbauer and Oechel 1989). In our study *D. octopetala* had the second highest rate of APS, 11.51 ± 1.78 ΠmolCO$_2$ m^{-2}s^{-1}. Other researchers have observed similar or higher rates for *D. octopetala* (McGraw 1987, Wookey et al. 1995, Welker et al. 1997) and the rates fall within the same range of those recorded in other studies at the same site (Chapters 2 and 3).

D. octopetala is usually classified as an evergreen dwarf shrub (e.g., Welker et al. 1993, Arft et al. 1999), however it is not a 'true' evergreen. Welker et al. (1997) define *D. octopetala* leaves as 'wintergreen', meaning that chlorophyll is broken down in the fall and the leaves become brown. These same leaves become photosynthetic the following spring and are usually functional for two years, after which they stay attached to the plant for another two to four years. By the definition of Welker et al. (1997) the leaves of *D. octopetala* are similar to evergreen species in that they are photosynthetically active for multiple years. However, the photosythetic rates observed in this study and others (e.g. Wookey et al. 1995, Welker et al. 1997) are higher than have been observed for other evergreen species (e.g. Oberbauer and Oechel 1989), suggesting that the photosynthetic strategy of *D. octopetala* is different from 'typical' evergreen species and more similar to deciduous shrubs.

Stomatal Density

We observed distinct species differences in stomatal density, ranging from 15.4 ± 0.8 mm^{-2} for *P. frigidus* to 47.9 ± 2.5 mm^{-2} for *S. reticulata* (Table 4-3). The only growth form with significant differences between species within the group was the deciduous shrubs; *S. reticulata* had a significantly higher stomatal density than both *S. arctica* and *S. polaris*. While there were also significant differences between growth forms, there was some overlap as the graminoids were not significantly different from either the deciduous shrubs or the perennial forbs. Due to the small number of species used in our study, the high density of *S. reticulata* and the low density of *P. frigidus* may have determined the significant difference between deciduous shrubs and perennial forbs.

Five of the eight study species were hypostomatous and three were amphistomatous. While it is more common for lowland species to be either hypostomatous or have a very low adaxial/abaxial ratio of stomatal density, it has been found that adaxial stomatal density increases and abaxial density decreases with elevation (Körner et al. 1986, Körner 1999). While the adaxial/abaxial ratio was relatively low for *P. bistorta*, 0.22, *S. polaris* and *E. latifolium* had higher frequencies, 0.78 and 1.13, perhaps indicating an anatomical adaptation to life at high elevation.

Comparison with Literature Values

Based on rates of photosynthesis reported in the arctic and alpine literature, perennial forbs had the highest rates of APS, followed by graminoids, deciduous shrubs and evergreen shrubs, respectively. Based on pooled values, significant differences existed between forbs and graminoids and forbs and deciduous shrubs. Rates for

deciduous and evergreen shrubs were very similar (11.3 $\pm$ 0.8 μmol m^{-2} s^{-1} and 11.2 $\pm$ 1.80 μmol m^{-2} s^{-1}, respectively). Based on the higher leaf turnover rate and shorter period of photosynthetic activity of deciduous shrubs (Arft et al. 1999), as well as the results of the study by Oberbauer and Oechel (1989), we expected deciduous shrubs to have a higher rate of APS than the evergreen shrubs. The evergreen rates cover a large range of values, 6 to 16 μmol m^{-2} s^{-1}, with the three highest rates belonging to *D. octopetala*. As we found with our own study, this suggests *D. octopetala* should not be included as an evergreen shrub in measurements of photosynthesis. If we removed *D. octopetala* from the evergreen shrub category, the average rate was 7.3 $\pm$ 1.0 μmol m^{-2} s^{-1}, similar to the results of Oberbauer and Oechel (7.7 $\pm$ 0.8 μmol m^{-2} s^{-1}) (1989).

While the results of the literature survey suggested that there was a general pattern in photosynthetic rates for the different growth forms, there was considerable overlap (Fig. 4-2). The perennial forbs had significantly higher rates than the graminoids and deciduous shrubs, however the range of values within each growth form was quite large. These results are consistent with those of Oberbauer and Oechel (1989), Körner (1999) and our study, suggesting that on its own, photosynthetic rate does not appear to be a reliable indicator of functional group.

Singsaas et al. (2001) cautioned against comparing photosynthetic values from the literature. They compared reported values of photosynthetic quantum yield from different studies reported in the literature with two large comparative studies and their own measurements. While several authors have reported variation among plant species of widely diverse origins (Bongi and Long 1987, Ögren and Evans 1993), Singsaas et al. (2001) attributed the wide range of values in the literature to data collection and analysis

techniques. These results also emphasize the importance of measurement and data analysis protocols in photosynthesis studies. Our literature survey results are certainly influenced by including studies using different techniques or equipment, and for this reason we avoided comparing absolute values obtained in our study to those in the literature, but rather emphasize the relationships between the different species and growth forms.

We included both alpine and arctic studies in the survey because there are relatively few alpine studies, particularly at high latitude sites like our own, and this may have introduced additional variation although we did not look at this issue explicitly (Fig. 4-2). For the reasons stated above we felt that a formal analysis using data from different studies (and different methods) was not appropriate. In addition to low temperatures during the growing season, different limiting factors exist for arctic and alpine plants. Low CO_2 partial pressure and strong winds are important limiting factors in alpine sites while arctic soils are generally more nutrient-limited than alpine soils (Billings and Mooney 1968, Bliss 1971). The nitrogen content of alpine plants also appeared to be higher than plants from Arctic sites (Körner et al. 1986), which could result in higher rates of photosynthesis for some species. However, this was not evident in the data we compiled (Fig. 4-2).

General Discussion

For functional groups to be useful in plant ecology the same grouping of species needs to describe vegetation responses to the environment, as well as vegetation effects on ecosystem processes (Chapin et al. 1996). The system of functional groups commonly

used for tundra plants is attractive because there are relatively few groups and it is easy to assign plants to these groups as they are based on growth form. Chapin et al. (1996) used traits expected to influence ecosystem processes, including photosynthetic rates, to validate the appropriateness of these functional groups. Studies in other ecosystems have also found physiological similarities between ecologically-defined groups. For example, a study of twenty-one tropical tree species in a Panamanian forest found that trees could be classified into functional groups of successional status based on measurements of physiological traits (steady-state leaf-level photosynthesis, saturated photosynthetic rates and leaf nitrogen concentration) (Ellis et al. 2000). We examined two easily measurable traits related to photosynthesis to determine if species within the traditional tundra functional groups were similar. We found little distinction between and few similarities within functional groups in measurements of stomatal density. However, species of the same functional group were similar in apparent photosynthesis, with the exception of evergreen shrubs. Still, these measurements did not necessarily separate the species into their functional groups as there was some overlap between species of different groups.

Photosynthesis is only one aspect of plant physiology and is not necessarily directly related to growth (Körner 1999). A combination of factors, including photosynthesis, respiration and fractionation of assimilates into autotrophic and heterotrophic structures, are important in predicting growth. By measuring only two factors it appears that we simplified things to a point where distinctions between functional groups, if they exist, could not be made.

The surprisingly high rate of *D. octopetala* was more similar to the forbs and deciduous shrubs than to the evergreen shrub *C. tetragona*. In the study by Chapin et al.

(1996) *Dryas* was grouped with evergreen shrubs, however their analysis recognized it as distinct from its functional group. Rather than 'forcing' *D. octopetala* into one of the four established functional groups, it could be classified as a unique functional group. The correct classification of *D. octopetala* is important at our site since it is one of the most abundant species. Classifying it in the wrong functional group could have serious implications for predictions about community response to change.

A balance between species-specific studies and those using coarse functional groups needs to be found. One way of doing this is by conducting a detailed study of the dominant species in a community while defining the remainder by functional groups. A study by Wohlfarht et al. (2001) took this approach for a vegetation-atmosphere CO_2 and energy exchange model for mountain grasslands. They then replaced physiological information at the species level with generic physiological parameters for the three main functional groups (forbs, graminoids and dwarf shrubs) and found that the latter produced acceptable results. This study demonstrates that making replacements is possible as long as a representative database of the site vegetation exists. Unfortunately such databases are rare, making it difficult to validate models.

In the current literature, there is a de-emphasis on defining functional groups based on static measurements and observations. Instead, recent papers have argued that if predictions are to made about response to change, functional groups should be defined based on these responses (e.g. Smith et al. 1993, Wilson 1999, Dyer et al. 2001). Dyer et al. (2001) caution against using 'static' measurements of morphology or one-time measurements of physiology to identify plant functional groups for use in making predictions about response to environmental change.

Dormann and Woodin (2002) used the functional groups of Chapin et al. (1996) in a meta-analysis of the results of arctic field experiments simulating climate change. While a few trends emerged, overall they found that use of the traditional plant functional groups was unsatisfactory for generalization. Dormann and Woodin (2002) concluded that a better alternative to the traditional functional groups would be functional groups based on species responses to environmental manipulations (e.g. Dyer et al. 2001). The results of our climate change study (Chapter 2) would not necessarily be predicted by the conclusions of Dormann and Woodin's analysis. For example, while they found significant physiological responses to both fertilization and elevated temperatures, we only observed one significant physiological response to elevated temperature and none for fertilization. Their meta-analysis also shows a strong response of grasses to fertilization, consistent with the observation of an increase in graminoids in nitrogen addition plots at our site (Hik and Koh, in prep). However, the significant decrease in the evergreen shrub *D. octopetala* at our site is not consistent with their results.

The use of growth forms as plant functional groups for predictions of responses to herbivory may not be appropriate either. Mechanisms of herbivory tolerance vary widely even among similar growth forms (Chapter 3, McIntire and Hik 2002). For example, in response to mammalian herbivory, we observed a delay in end of season senescence for the graminoid *C. consimilis* and an increase in leaf births for the 'wintergreen' dwarf shrub *D. octopetala.*, while McIntire and Hik (2002) reported a higher rate of leaf production in a graminoid, *Kobresia myosuroides*, and a forb, *Oxytropis nigrescens,* and a delay in leaf senescence in a forb, *Erigeron humilis*, at the same site. It is unlikely that

we would have predicted our results based on the earlier study if we were to make hypotheses based on growth form alone.

The challenge in defining plant functional groups is to capture enough detail to represent the vegetation accurately, while still simplifying the structure sufficiently to develop reasonably accurate ecosystem-level models. Epstein et al. (2001) conducted a study examining the effects of different levels of aggregation of arctic plant species for modeling purposes and found that the level of aggregation had an impact on the model prediction outcome. They suggest that aggregating vegetation to levels less detailed than plant functional groups will have strong implications for regional-scale modeling of vegetation dynamics and carbon cycling. The results of our study and others (e.g. Oberbauer and Oechel 1989, Epstein et al. 2001, Dormann and Woodin 2002) suggest that using the traditional functional groups will not be sufficient for making ecosystem predictions. Rather, a combination of these traditional functional groups along with detailed studies of regionally important species will perhaps provide clearer predictions. If this approach is taken, as changes in community composition occur the focal study species will also have to change.

Overall, our results indicated that growth form alone is not a good indicator of photosynthetic rates or stomatal densities and patterning of alpine plants. Other characteristics, such as growth rate or response to experimental treatment (Dyer et al. 2001) may provide a more consistent means of defining functional groups for alpine plants. Functional groups are useful in many applications, but it is still unclear how they can best be defined.

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Table 4-1. Growth form of ten alpine species from the Ruby Range, Yukon.

Growth form	Representative species
Perennial forb	*Epilobium latifolium* L., *Petasites frigidus* (L.) Fries, *Polygonum bistorta* L.
Graminoid	*Carex consimilis* Holm, *Festuca altaica* Trin.
Deciduous shrub	*Salix arctica* Pall., *Salix polaris* Wahlenb., *Salix reticulata* L.
Evergreen shrub	*Cassiope tetragona* (L.) D. Don, *Dryas octopetala* L.

Table 4-2. ANOVA summary for APS, fluorescence parameters and stomatal density.

Parameter	Species		Growth form	
	$F_{(numdf,dendf)}$	*P*	$F_{(numdf,dendf)}$	*P*
APS (mg CO_2 dm^{-2} h^{-1})	**$5.16_{(9,40)}$**	**<0.0001**	**$4.88_{(3,16)}$**	**0.0135**
F_v/F_m	$1.48_{(9,33)}$	0.1956	$1.50_{(3,16)}$	0.2531
F_v'/F_m	$0.99_{(9,31)}$	0.4656	$1.50_{(3,16)}$	0.2526
Φ_{PSII}	$1.76_{(9,31)}$	0.1168	$0.38_{(3,16)}$	0.7674
QP	$1.41_{(9,30)}$	0.2271	$0.14_{(3,16)}$	0.9371
NPQ	$1.93_{(9,30)}$	0.0863	$1.45_{(3,16)}$	0.2663
Stomatal Density	**$10.56_{(7,7)}$**	**0.0030**	**$4.21_{(2,12)}$**	**0.0411**

Table 4-3. Stomatal density and distribution of ten alpine plant species, representing four growth forms.

Species	Growth form	Abaxial (Ab) (mm^{-2})	Adaxial (Ad) (mm^{-2})	Ad/Ab
Carex consimilis	Gr	45.5±2.9	---	---
Festuca altaica	Gr	61.7±9.2	---	---
Salix arctica	DS	53.4±3.6	---	---
Salix polaris	DS	30.0±1.4	23.4±1.9	0.78
Salix reticulata	DS	95.8±5.0	---	---
Epilobium latifolium	F	23.3±1.6	26.4±1.8	1.13
Petasites frigidus	F	30.8±1.6	---	---
Polygonum bistorta	F	28.6±1.6	6.4±0.5	0.22

Ad/Ab, adaxial/abaxial ratio of stomatal density; results based on 2 investigated leaves per species (4 counts per leaf); Gr = graminoid, DS = deciduous shrub, F = forb

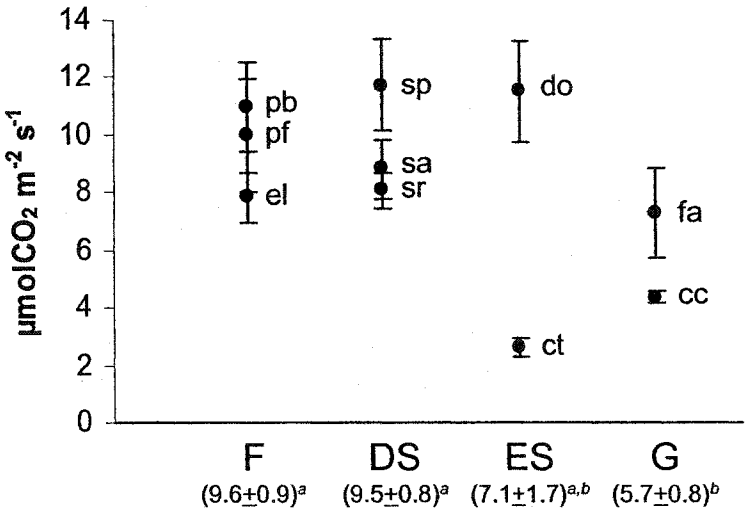

Fig. 4-1. Apparent photosynthesis (APS) for 10 alpine plant species (pb (*Polygonum bistorta*), pf (*Petasites frigidus*), el (*Epilobium latifolium*), sp (*Salix polaris*), sa (*Salix arctica*), sr (*Salix reticulata*), do (*Dryas octopetala*), ct (*Cassiope tetragona*), fa (*Festuca altaica*), cc (*Carex consimilis*)) and by growth form (F (perennial forbs), DS (deciduous shrubs), ES (evergreen shrubs), G (graminoids)). Values are means ± 1SE of 5 replicates per species. Values in brackets are means ± 1SE for each growth form. Letters indicate significant differences between growth forms (LSD, $P<0.05$, unadjusted).

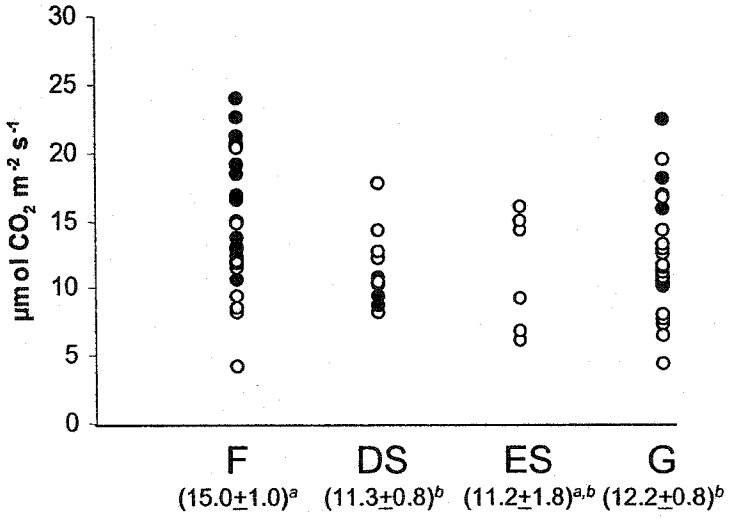

Fig. 4-2. Maximum photosynthetic rates of arctic (○) and alpine (●) species reported in the literature. F (perennial forbs; n=27), DS (deciduous shrubs; n=12), ES (evergreen shrubs; n=6) and G (graminoids; n=25). Values in brackets are means ± 1SE for each growth form. Letters indicate significant differences between growth forms (LSD, $P<0.05$, unadjusted).

CHAPTER 5

General Discussion

This thesis reported on the responses of several alpine plant species to climate change and herbivory in the southwest Yukon. Measurements of photosynthesis and within-season leaf demography were made to examine physiological and growth responses. I also undertook a study of physiological and structural similarities among ten alpine plant species to look for characteristics associated with growth form and functional groupings. While similar studies have been conducted in the Arctic and mid-latitude alpine regions, this is one of the first studies in a sub-arctic alpine area.

While this study has examined three distinct topics, there were two common objectives that unified the chapters: an examination of the relationship between two ecosystem processes, photosynthesis and within-season leaf demography and an examination of whether or not species responses could be generalized by growth form for making future predictions about changes and/or responses of alpine species to disturbances.

The Relationship Between Ecosystem Processes

Ecosystem processes respond to disturbance at different time scales. This means that not only the magnitude but also the direction of the response can change over time (Shaver et al. 2000). In addition, buffers may exist at certain levels of response, therefore short-term physiological responses are not always good indicators of response to the environment (Chapin and Shaver 1996). For example, an increase in photosynthesis may

not necessarily result in an increase in size, depending on the factors limiting growth. I took measurements at two scales, within-season leaf demography and photosynthesis, in response to a) warming and/or nitrogen addition and b) herbivory, to see if there was any correlation between these two measurements. The results show few similarities between the two and suggest that it is not possible to predict one measurement based on the other (Table 5-1). Rather, the combination of these two measurements provide a clearer picture of how the study species are dealing with these stresses than if only one of demography or photosynthesis was measured.

In the climate change study, responses in demography measurements were observed for all four study species. *Carex consimilis* Holm cumulative leaf births was increased in the +T+N plots; *Dryas octopetala* L. rate of leaf births was higher in the +N and +T+N plots at the end of the growing season; *Polygonum bistorta* L. rate of leaf deaths was higher in the +N and +T+N plots at the end of the growing season and; an increase in leaf length for *Salix reticulata* L. in +N and +T+N plots was observed. I only observed one significant effect on APS: *P. bistorta* plants in the +T plots had higher rates than those in the three other plots. Six years following the initiation of climate change manipulations responses appear to be at the leaf level rather than the physiological level.

The mechanisms for dealing with high rates of tissue loss to herbivory were more apparent in demographic measurements, however the photosynthesis data complemented the demographic results. We found no evidence for compensatory photosynthesis, however, when photosynthesis was combined with demography, an indication of possible mechanisms associated with tolerance were found.

Table 5-1. Species-specific responses to warming and/or nitrogen addition (climate change) and herbivory, in within-season leaf demography (demography) and rates of apparent photosynthesis (APS). CLB – cumulative leaf births, CLD – cumulative leaf deaths, RLB – recent leaf births, RLD – recent leaf deaths and LL – leaf length.

C. consimilis

	Climate Change		Herbivory	
	Predictions	Observations	Predictions	Observations
Demography	↑ CLB in +T+N, +N & +T	+T+N > control, +T & +N	Either ↑ CLB OR	↓ CLD for plants grazed in the current season
	↓ CLD in +T+N, +N & +T	---	↓ CLD for historically grazed plants	
	↑ LL in +T+N, +N & +T	---		
APS	↑APS in +T+N, +N & +T	No sig. diff.	↑APS for plants grazed in the current season	No sig. diff.

D. octopetala

	Climate Change		Herbivory	
	Predictions	Observations	Predictions	Observations
Demography	↓ CLD in +T+N, +N & +T	RLB: +T+N & +N > +T & control (end of season)	Either ↑ CLB OR ↓ CLD for historically grazed plants	↑ CLB for plants grazed in the current season
APS	No effect	---	↑APS for plants grazed in the current season	↓APS for plants grazed in the current season

P. bistorta

	Climate Change	
	Predictions	Observations
Demography	↑ CLB in +T+N, +N & +T	RLD: +N & +T+N > +T &
	↑ LL in +T+N, +N & +T	control (end of season)
APS	↑APS in +T+N, +N & +T	+T > +T+N, +N, control

S. reticulata

	Climate Change	
	Predictions	Observations
Demography	↑ CLB in +T+N, +N & +T	LL: +T+N, +N > +T, control
	↑ LL in +T+N, +N & +T	
APS	no effect	control ≥ +T ≥ +N ≥ +T+N (g^{-1} chl only)

A delay in end of season senescence was observed for the sedge *C. consimilis* in response to herbivory, however, no difference in rates of APS was observed. The combined knowledge that grazed plants had photosynthetic tissue later in the growing season and maintained rates of photosynthesis equal to those of ungrazed plants suggests that these two mechanisms provide the plants with a means to replace tissue lost to herbivores.

Lower rates of APS were observed for the 'wintergreen' shrub *D. octopetala*, however, grazed plants also had higher rates of leaf births. The higher leaf births indicate that *D. octopetala* is able to compensate for lost tissue. A longer-term study (across the entire growing season) is needed to determine if rates of APS are lower across the entire growing season or only following herbivory.

Using Functional Groups to Make Predictions

Attempting to predict the responses of ecosystems to disturbances such as climate change or herbivory is a difficult task facing biologists. One of the major obstacles is the complexity of ecosystems. It is impossible to look at every species in a community and incorporate this level of complexity into a model. Using plant functional groups is an attractive way of overcoming this problem. However, the results of the species survey suggest that care must be taken in doing this.

Little evidence was found for similarities between functional groups for rates of APS. Inherent physiological pathways are thought to be similar among species of the same growth form (Billings 1992) and due to these underlying similarities it is thought that species of the same growth form will respond to change in a similar manner (Arft et

al. 1999). Photosynthetic rate was one of the parameters included in the classification of Chapin et al. (1996). This classification has been used for building models predicting the response of ecosystems to disturbance, particularly climate change (e.g. Epstein et al. 2001, Wohlfahrt et al. 2001). My study suggests that caution should be taken when doing this. I found that species of the same growth form were not necessarily similar in rates of APS.

The largest difference was found in the evergreen dwarf shrub category as *D. octopetala* had much higher rates than would be predicted for an evergreen species. This indicates the necessity of studying the important or dominant species in an ecosystem before models are to be made for that region. Attempts are being made to come up with general patterns of response to climate change (e.g. Arft et al. 1999, Dormann and Woodin 2002), however few general patterns have emerged, suggesting the importance of considering the unique abiotic and biotic characteristics of different sites. It appears that the most appropriate way of modeling possible future scenarios is through a combination of detailed species-specific responses to potential disturbances (such as our climate change study) for the dominant species, with more simplified groupings for the remaining species (e.g. Wohlfahrt et al. 2001).

Future Research

It is predicted that as alpine treeline advances, alpine ecosystems will be forced into smaller and smaller areas. This will have impacts on alpine plant species as well as the herbivores that rely on them as a food source. Extinctions are predicted to occur but it is unknown which species will be most affected (Murphy and Weiss 1992). Studies

have shown that alpine plant species migrations occur slowly (e.g. Grabherr et al. 1995) and that shorter-term changes will affect community composition (Arft et al. 1999). Several studies have looked at how climate change will affect tundra herbivores (e.g. Walsh et al. 1997, Van der Wal 2000) but this remains an understudied topic. An understanding of how the vegetation will be affected is necessary for determining how herbivores will be affected.

A logical next step would be to combine the climate change and herbivory studies. The herbivory study revealed that grazed and ungrazed plants of the same species differ in photosynthesis and/or within-season leaf demography. Herbivory is an important process impacting vegetation at the study site, therefore it is important to consider how both grazed and ungrazed plants will be affected by climate change. Studies have shown that both climate change and herbivory can influence community composition (e.g. climate change: Chapin and Shaver 1996, Robinson et al. 1998, Arft et al. 1999, herbivory: Bazely and Jefferies 1986, Huntly 1987, Tolvanen and Henry 2000). How these two disturbances will work together to influence alpine plant communities is less certain. For example, an increase in graminoids, particularly grasses, is predicted for increased nitrogen addition (Dormann and Woodin 2002). An increase in graminoids will result in a decrease in forbs as grasses shade forbs, reducing photosynthetic rates. However, if these plants were grazed, grass cover would be reduced, resulting in a reduction in shading.

Designing studies to investigate the effects of climate change on tundra plant species that include the impacts of herbivores is a challenge facing ecologists. ITEX has recognized the need for more studies including herbivory (Henry and Molau 1997). As

there are problems with simulating herbivory by clipping plants (Baldwin 1990, Paige 1999), climate change experimental plots need to be established in areas that experience regular grazing. Detailed studies of this sort will help to expand the current knowledge of how tundra systems will be affected by climate change. As herbivory is an important factor affecting many tundra systems, it must be fully integrated in future studies of climate change.

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APPENDIX

Extracting chlorophyll under field conditions: how stable is dimethylsulfoxide (DMSO)?

Introduction

Several methods are commonly used to measure chlorophyll in plants. There are many reasons why particular extraction methods are used, but this inherent methodological variation may create several problems in making comparisons between species, or different studies. Potential biases in chlorophyll extraction may be due to solvent type (Stauffer et al. 1979, Speziale et al. 1984, Porra et al. 1989, Garcia and Nicolas 1998a), solvent impurity (Garcia and Nicolas 1998b), degree of leaf maceration (Shinano et al. 1996), tissue type (Shinano et al. 1996), and the equations used to calculate chlorophyll concentrations (Barnes et al. 1992, Wellburn 1994).

While controlled chlorophyll extraction under laboratory conditions may reduce methodological problems (e.g. Thayer and Björkman1990, Garcia and Nicolas 1998a, Rosevear et al. 2001), a robust and reliable field method is also required in many ecological studies. Under field conditions the ideal solvent would be non-toxic, relatively stable, and capable of complete and unbiased extraction of whole tissue across a range of species and environmental conditions. While 80% acetone is commonly used to extract chlorophyll, this method requires maceration of plant tissue and centrifugation before measurement. Two extraction methods that do not require these initial processing steps, and are therefore more suitable for field conditions, are incubation in N,N-

dimethylformamide (DMF: Moran and Porath 1980, Yoder and Deley 1989) and dimethylsulfoxide (DMSO: Hiscox and Israelstam 1979, Barnes et al. 1992).

DMSO has been widely used for chlorophyll extraction from higher plants (Hiscox and Israelstam 1979, Barnes et al. 1992, Wellburn 1994), lichen (Ronen and Galun 1984, Barnes et al. 1992), mosses (Alpert 1984) and aquatic plants (Shoaf and Lium 1976, Spencer and Ksander 1987). It is an attractive solvent for chlorophyll extraction because instead of grinding and centrifuging tissue, small pieces are placed in the solvent until the tissue clears. The DMSO method allows for a large number of samples to be prepared and analyzed quickly. An additional advantage is that spectrophotometer readings can be made on extracts stored in a cool, dark place for up to seven days (Hiscox and Israelstam 1979, Barnes et al. 1992). We examined the effect of incubation temperature, incubation duration, and subsequent storage on vascular plant chlorophyll extraction using two solvents, DMSO and 80% acetone. Our primary objective was to establish the conditions under which DMSO may be suitable for chlorophyll extraction in field studies.

Materials and Methods

We collected fully expanded leaves of Prunus *virginiana* (chokecherry) trees growing on the University of Alberta grounds, and *Fragaria vesca* (alpine strawberry), *Helianthus annuus* (sunflower), and two graminoids (*Andropogon gerardii:* big bluestem; and *Cymbopogon citrates:* lemongrass) from the greenhouse. All material was processed immediately in the laboratory using methods similar to those employed under subarctic alpine field conditions (see Hik et al. 2001 for a site description).

DMSO Method

A 20 mg sample of leaf tissue was cut into small (4-16 mm^2) pieces and placed in a vial with 7 mL DMSO. Five replicates of each species were placed in waterbaths maintained at 25°C, 30°C or 40°C, temperatures chosen to bracket the range that can be maintained at our field sites. In a separate set of incubations, the effect of the leaf mass to DMSO volume ratio on extraction was examined for *P. virginiana* by extracting different amounts of tissue (20, 40, 60, 80 and 100 mg) in 10 mL of DMSO for four hours at 40°C.

Acetone Method

A 20 mg sample of leaf tissue was ground in a pestle and mortar in 5 mL acetone with fine sand. Extracts were adjusted to a final volume of 10 mL and centrifuged at 663 x *g* for 5 minutes. Five replicates per species were prepared.

Spectrophotometry

Chlorophyll extract was transferred to a cuvette and spectrophotometer readings were made at 649 and 665 nm (DMSO) and at 646 and 663 nm (acetone) on a field spectrophotometer (Turner, Model 330). If OD values were higher than 0.7, the extract was diluted accordingly (Hiscox and Israelstam 1979). Readings were taken immediately after extract preparation (acetone only), as well as after 2, 4, 7, 24 and 48 hours. Acetone extracts were stored at room temperature between readings and DMSO extracts continued to incubate. After the 48 hour reading, extracts were stored at 4°C in the dark, and

measured again after 1, 3 and 7 days. Samples were thawed (~20°C) and then refrozen after each reading.

Analysis

Chlorophyll concentrations were calculated using Wellburn's (1994) equations for a low resolution spectrophotometer, and were expressed as micrograms chlorophyll per 100 mg fresh mass (μgChl/100mgFM). For DMSO, $chl_a = 12.19A_{665}-3.45A_{649}$ and $chl_b = 21.99A_{649}-5.32A_{665}$, and for Acetone, $chl_a = 12.21A_{663}-2.81A_{646}$ and $chl_b = 20.13A_{646}-5.03A_{663}$. The effect of incubation temperature on DMSO extraction efficiency was determined for total chlorophyll ($a + b$), chl a, chl b, and chl a/b ratio using a 3-way repeated measures (RM) ANOVA, with Time as the repeated factor and Species and Temperature as the main effects. Effect of the method of chlorophyll extraction (Solvent: DMSO or acetone) was analyzed using a 2-way ANOVA with Species and Solvent as the main effects. Acetone extractions (Time = 0) were compared to DMSO extractions (Time = 7 or 24 hours depending on species). Effect of the duration of cold storage (0-7 days) was analyzed using a 3-way RM-ANOVA with Time as the repeated factor and Species and Solvent as main effects. The effect of the mass/volume ratio on chlorophyll levels was analyzed using a one-way ANOVA. Multiple comparisons were performed using unadjusted Least Square Means, and all statistical analyses were conducted using SAS 8.1 (SAS Institute Inc. 1999). Results are reported as means $\pm$ 1 S.E. Alpha was conservatively set at 0.005 because multiple tests were conducted.

Results

Using DMSO as the extraction solvent, *F. vesca* had the highest concentration of total chlorophyll (*a* + *b*), chl *a* and chl *b,* and *P. virginiana* and *C. citrates* had the lowest concentrations (Fig. 1). Using acetone, *F. vesca* also had the highest concentration of total chlorophyll (*a* + *b*), chl *a* and chl *b,* and *P. virginiana* and *H. annuus* had the lowest concentrations. Extraction in DMSO was essentially complete after 7 hours of incubation for *P. virginiana* and *H. annuus,* and after 24 hours of incubation for *A. gerardii, C. citrates* and *F. vesca* (Fig.1). There was no significant effect of Temperature (RM-ANOVA, all P-values > 0.2134) or mass/volume ratio (one-way ANOVA, all P-values > 0.07) on total chlorophyll (*a* + *b*), chl *a*, chl *b* and chl *a*/*b* using DMSO. Total chlorophyll (*a* + *b*), chl *a* and chl *b* concentration using DMSO were significantly lower than from acetone extraction for only one species, *C. citrates* (LS Means P-value < 0.0001).

During 7 days of cold storage there was a significant difference in total chlorophyll (*a* + *b*) and chl *b* between the two Solvents (RM-ANOVA, P < 0.003). As well, there was a significant effect of Time on all chlorophyll measures (RM-ANOVA, P < 0.005). Total chlorophyll (*a* + *b*) concentration of all five species decreased during the week of storage for the DMSO samples (Fig.1), and this was a result of changes in both chl *a* and chl *b* concentrations. For stored acetone samples, *C. citrates* and *F. vesca* total chlorophyll (*a* + *b*) changed significantly due to decreases in both chl *a* and chl *b* (Fig.1).

Discussion

The results obtained using the DMSO method were consistent with those obtained using the acetone method for four of the five study species. However, for *C. citrates* the results obtained using DMSO were significantly lower than those obtained using acetone for total chlorophyll (*a* + *b*), chl *a*, and chl *b*. The lower total chlorophyll (*a* + *b*) concentration of the graminoid *C. citrates* using the DMSO method suggests that temperatures below 40°C may not be sufficient for complete chlorophyll extraction from thick, highly cutinized leaves. Barnes et al. (1992) found that extracting chlorophylls from lichens at temperatures below 40°C resulted in lower total chlorophyll (*a* + *b*) concentrations, especially chl *b*, and higher chlorophyll *a*/*b* ratios. However, a higher temperature may still not be sufficient for complete extraction. Shinano et al. (1996) reported that maceration of graminoid leaf tissue was necessary for complete extraction using DMSO at an incubation temperature of 65°C. While tissue maceration prior to extraction may be necessary for graminoid leaves, our results also indicate that temperatures between 25-40°C were generally sufficient for complete extraction using DMSO. We also observed no effect of the mass to volume ratio for chlorophyll extracted in DMSO, suggesting that with appropriate dilution of concentrated samples, the initial mass of plant tissue is not important as long as sufficient tissue is used.

The equations used to calculate chlorophyll concentration might also influence the results because the absorption spectra of chl *a* and chl *b* are different in DMSO and acetone. Consequently, Arnon's (1949) absorption spectra and equations for acetone will give inaccurate results for DMSO (Barnes et al. 1992). Several studies (e.g. Inskeep and

Bloom 1985, Porra et al. 1989, Barnes et al. 1992, Wellburn 1994) have modified Arnon's equations, and most importantly, Wellburn (1994) compared high and low resolution spectrophotometers to conclude that both types of instruments have the potential to provide accurate results, as long as the proper equations are used. Wellburn's (1994) equations indicated significant differences exist for only one species (*C. citrates*) in our study, whereas Barnes et al. (1992) equations indicated significant results for four species.

In contrast with other studies (e.g. Hiscox and Israelstam 1979, Spencer and Ksander 1987; but see Alpert 1984), our DMSO extracts stored over one week were not stable. The decrease we observed could be a consequence of thawing and freezing samples each time readings were made. Barnes et al. (1992) also found that thawing and refreezing resulted in a decrease in chlorophyll concentration of lichen samples, suggesting that samples must be kept frozen while in storage and not allowed to thaw. This is a requirement that may present difficulties in some field situations where the temperature fluctuates above and below the freezing point of DMSO (18.5°C).

In field conditions with limited lab resources the DMSO method of extraction is simpler alternative to the acetone method. However, prior to using DMSO in a field study, its effectiveness under the prevalent field conditions should be assessed in the laboratory. Finding a solvent that is reliable under variable conditions will allow for the collection of information on chlorophyll concentration from remote sites where it is impossible to maintain a complete laboratory. Other methods which are effective on intact plant tissue, stable at low and variable temperatures, and do not require mechanical maceration or particle removal, including DMF (Moran and Porath 1980, Yoder and

Deley 1989), should be considered further. Instrumental methods may also be appropriate in some situations. For example, Schaper and Chacko (1991) reported a significant linear relationship between acetone extractable total leaf chlorophyll and optical densities of leaves estimated using a portable chlorophyll meter.

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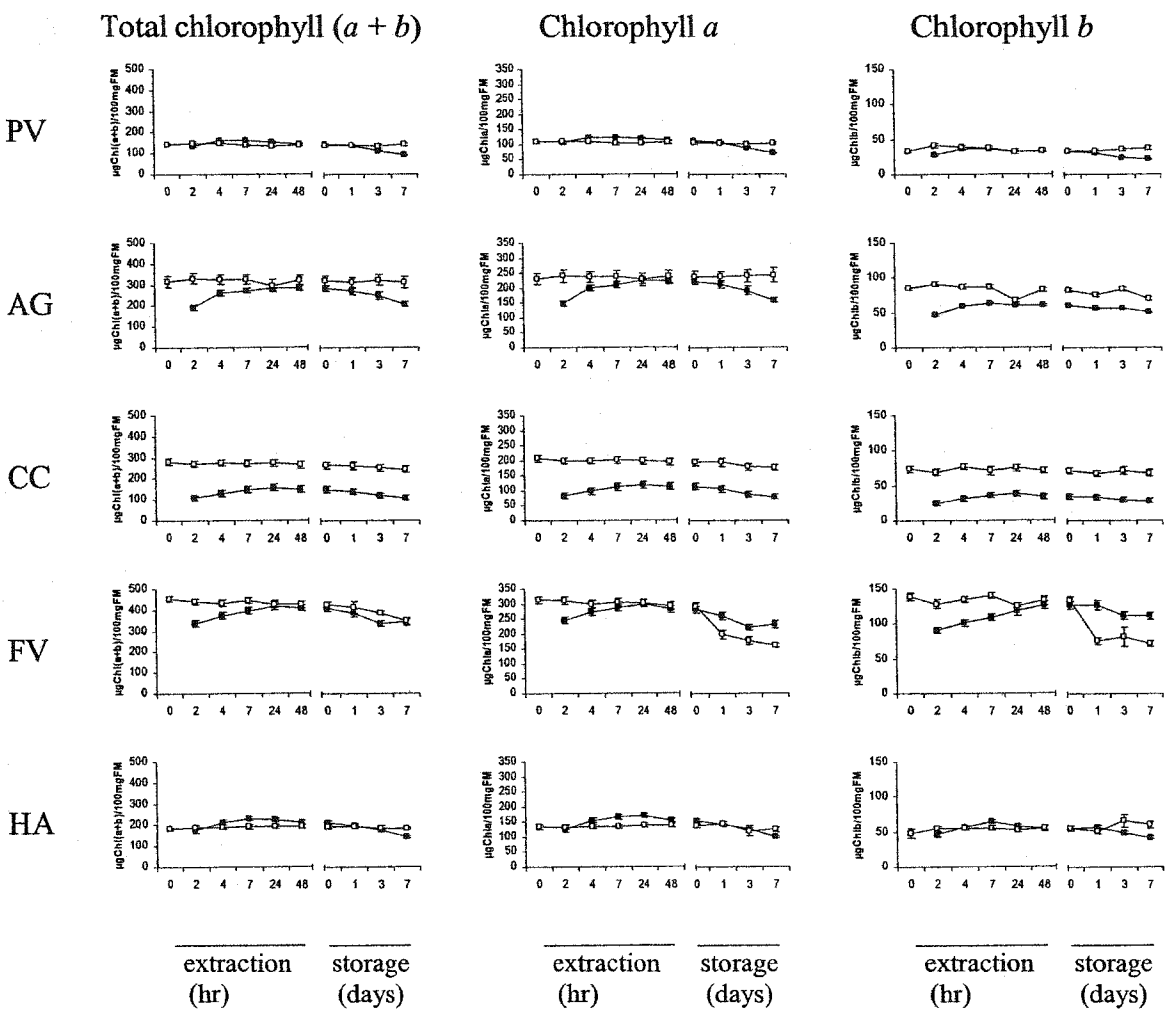

Fig. 1. Total chlorophyll (a + b), chlorophyll a and chlorophyll b concentration of *P. virginiana* (PV), *A. gerardii* (AG), *C. citrates* (CC), *F. vesca* (FV) and *H. annuus* (HA) extracted with DMSO at 40°C (solid) or acetone (open) over 48 hours and then following storage at 4°C in the dark for 7 days. Values are means ± 1SE (n=5).