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Effects of a short-term freeze on Sphagnum girgensohnii grown under different light-temperature regimes

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EFFECTS OF A SHORT-TERM FREEZE ON

SPHAGNUM GIRGENSOHNII GROWN UNDER DIFFERENT LIGHT-

TEMPERATURE REGIMES

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by

Sarah Jane Colby

2007

To: Interim Dean Mark Szuchman
      College of Arts and Sciences

This thesis, written by Sarah Jane Colby, and entitled Effects of a short-term freeze on Sphagnum girgensohnii grown under different light-temperature regimes, having been approved in respect to style and intellectual content, is referred to you for judgment.

We have read this thesis and recommend that it be approved.

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Florida International University, 2007

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DEDICATION

For my mom and my dad: together they embody all that is good in the world.

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ABSTRACT OF THE THESIS

EFFECTS OF A SHORT-TERM FREEZE ON SPHAGNUM GIRGENSOHNII

GROWN UNDER DIFFERENT LIGHT- TEMPERATURE REGIMES

by

Sarah Jane Colby

Florida International University, 2007

Miami, Florida

Professor Steven F. Oberbauer, Major Professor

During the short, snow-free growing seasons in the Arctic, sudden "cold snaps" or freeze thaw events (FTE) frequently occur when temperatures fall subzero for 24 to 72 h. Vascular plants exposed to FTE are often irreversibly damaged, but despite their importance, the responses of nonvascular plants to FTE have been little studied. I grew plants of *Sphagnum girgensonhii* under high and low light and temperature conditions to investigate whether pre-freeze conditions influence damage and recovery of this important moss species. Plants grown at low light and high temperature showed the greatest growth. Upon freezing they also showed irreversible physiological damage and the greatest reduction in growth. Furthermore, some growing conditions resulted in increased production of new branches that were lost during freezing. The findings of this study suggest that the responses of *Sphagnum* species to climate variation may be important for the structure of arctic plant communities.

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CHAPTER I

Global climate change and the genus *Sphagnum*: a review.

Sphagnum mires have been estimated to cover about 150 million hectares worldwide (Clymo and Hayward 1982), which equates to 120 billion metric tons of carbon (Longton 1992). Labeled as ecosystem engineers (Svensson 1995, Van Breeman 1995), *Sphagnum* mosses sequester nutrients that maintain these vast mires while providing a niche for many other acidophilic species. Predominately found in the higher latitudes, *Sphagnum*-dominated peatlands facilitate the highest rates of carbon sequestion in boreal and polar regions (Berense *et al.* 2001). This accumulation of carbon allows these regions to act as atmospheric CO_2 sinks (Gorham 1991) resulting from: 1) low belowground temperatures; 2) acidic conditions; 3) high soil moisture; 4) recalcitrant litter.

Here I highlight the importance of the genus *Sphagnum* to higher-latitude ecosystems. The role of *Sphagnum* mosses under climatic change is reviewed, outlining the current literature that examines the effects of altered environmental conditions on the growth and physiology of these bryophytes. Identifying the ecophysiological response of the genus *Sphagnum* is critical to understanding the ecosystem response of *Sphagnum*-dominated landscapes in this dynamic climate era.

Climate change in Sphagnum-dominated ecosystems.

While climate change is predicted to occur globally, climate models forecast the high latitudes to be the most severely impacted (IPCC 2001, Maxwell 1992). The Arctic, an extreme environment sensitive to climatic change (Maxwell 1992), is at considerable

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risk of vegetation changes under warmer conditions. Evidence is now accumulating that such changes are already occurring (Walker *et al.* 2006, Hinzman *et al.* 2005, Kulman 2002, Serreze *et al.* 2000). Perhaps even more critical, the potential of the Arctic to act as an additional source of carbon because of increased thawing of the permafrost, which leads to increased microbial activity and decomposition rates, has global repercussions (Marion *et al.* 1997, Oechel *et al.*1993, Koprivnjak & Moore 1992).

Research that examines physiological responses of arctic tundra plants may provide insight as to the consequences of climatic change in the northern latitudes. *Sphagnum* mosses compose a significant portion of the arctic vegetation (Hobbie *et al.* 2000; Walker *et al.* 1989). Hastings *et al.* (1989) estimated that *Sphagnum* accounts for approximately 27.6% of live plant biomass in the low Arctic, indicating the importance of understanding the ecosystem role of this genus.

Sphagnum: key player in a carbon shift?
Sphagnum and other mosses not only act as ecological indicators of the intensity of climatic changes (Andrus *et al.* 1992), but may also be critical regulators of the impact that climate change has on tundra. *Sphagnum,* accounting for a substantial amount of the tundra understory, acts as the interface between aboveground climatic variables and belowground processes. If *Sphagnum* growth responds positively to climate change, then the potential of the melting permafrost layer acting as a carbon source may be minimized. Because of its ability to insulate the permafrost layer and to store carbon (Vitt *et al.* 1994), *Sphagnum* may thereby act as a negative feedback that will compensate for a change in the carbon balance. Oechel & Vourlitis (1994) proposed a similar argument that the Arctic may at first act as a source of carbon, but then selectively adapt to the

increase in available nutrients, ultimately transforming back into a carbon sink. Likewise, if *Sphagnum* growth and photosynthetic rates are negatively impacted by climate change, then *Sphagnum* could act as a positive feedback of climate warming. This positive feedback could exacerbate carbon release by speeding up peat decomposition rates and lowering permafrost depths. Further research needs to be geared towards understanding the photosynthetic and growth responses of *Sphagnum* to the multiple climatic changes that are predicted to occur (Hobbie *et al.* 2000).

Succession as a climate regulator.

The ability of *Sphagnum* to dominate a landscape has prompted discussion of the role of *Sphagnum* in succession. Traditionally, *Sphagnum* species are thought to be responsible for the succession process of converting water bodies to dry land (Longton 1992), allowing vascular plants and then forests to colonize the area. In the Arctic where the climate is severe, *Sphagnum* may be an example of Muller's (1952) auto succession, where climatic factors hold competition to a minimum and thereby allow *Sphagnum* species to maintain occupancy in the community. Additionally, some researchers have described the role of *Sphagnum* as that of an ecosystem engineer (Heijmans *et al.* 2001; Svensson 1995; Van Breemen 1995), where *Sphagnum* is sequestering nutrients to maintain its status rather than creating an environment more suitable to vascular plants that would facilitate the succession process. During a fertilization experiment, Svensson (1995) found that the vascular rosette plant, *Drosera rotundifolia*, responded with vertical growth only when *Sphagnum* challenged it with its own vertical growth, which may indicate that vascular bog plants are not adapted to the advancing of the dominating

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Sphagnum. Alternatively, Ohlson *et al.* (2001) recently reported that *Sphagnum* mosses may be out-competed by the early succession of Scot pine. These conflicting findings suggest that *Sphagnum* in the Arctic is at a growth-rate equilibrium with its present vascular competitors, but this equilibrium may be at considerable risk if climate warming shifts the growth balance among species or allows the introduction of superior competitors. Knowledge of how *Sphagnum* growth rates vary under a variety of climate changes is crucial to understanding how climate change may affect the composition of the arctic landscape.

Response of Sphagnum to climate change: present understanding

Because of its abundance and role in the formation of mires, *Sphagnum* has long intrigued bryologists, and fundamental studies of *Sphagnum* physiology have been slowly accumulating for a century. In recent decades, studies have been geared towards examining the growth and physiology of *Sphagnum* under altered environmental conditions to gain insight into vegetative response to a range of habitats and recently to potential climate change scenarios (Mitchell *et al.* 2002, Searles *et al.* 2002, Berendse *et al.* 2001).

One global change already occurring at an exponential rate since the on-set of the western industrial revolution is the increase of atmospheric CO_2, which has gone from 280 ppm in the late 1800's to current 379 ppm by 2005, and is expected to climb throughout this century (IPCC 2007). An increase in atmospheric CO_2 may increase growth of some *Sphagnum* species. Heijmans *et al.* (2001) found that *S. magellenicum* length growth was correlated with increased CO_2 levels. Jauhiainen *et al.* (1998) offer

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further support demonstrating a correlation between increased atmospheric CO_2 and an increase in biomass, density and individual growth length of *S. angustifolium* and *S. warnstorfii*. This increase in growth of *Sphagnum* due to atmospheric CO_2 could be compounded as well by additional CO_2 availability in moss mats originating belowground as the permafrost layer thaws. Berendse and colleagues (2001) found, however, that an increase in atmospheric CO_2 did not influence the growth of *Sphagnum* in four high latitude locations across Western Europe. These conflicting studies can be reconciled by the findings that the response of *Sphagnum* species to increases in CO_2 can differ regionally, a further consideration that may be important when estimating the changing carbon budgets.

Further, interspecific growth-rate differences within the genus may also occur, which could lead to genus-level community structure shifts in peatland regions (Mulligan & Gignac 2002, 2001). Increases in atmospheric CO_2 may cause broad shifts in plant community structure by altering existing competition dynamics. Keeling *et al.* (1996) showed that a shift in the annual CO_2 concentration pattern provides evidence that the growing season is lengthening, especially in the Arctic.

In addition to carbon, the melting permafrost layer may also release stored nitrogen, commonly thought to be a limiting factor for Arctic plants (Aldous 2002, Gunnarson & Rydin 2000). A change in belowground nitrogen may influence existing interspecific competition between and among vascular and non-vascular species. Research has suggested that *Sphagnum* growth (Berendse *et al.* 2001; Heijmans *et al.* 2001; Gunnarsson & Rydin 2000; Hogg *et al.* 1995) and biomass (Van DerHeijden *et al.* 2000) decreases with increased available nitrogen. Thus a melting permafrost layer

increasing available nitrogen may cause *Sphagnum* to act as a source of carbon as the tundra warms. Changes in hydrology may also affect the ability of *Sphagnum* to retain N (Aldous 2002).

Climate change is anticipated to alter precipitation patterns worldwide (IPPC 2007, Dore 2005). The level of the soil water table is directly influenced by changes in the precipitation and temperature. In the Arctic, Kane *et al.* (1992) argue that thawing of the permafrost layer via global warming will lower the water table level, thereby lowering the moisture level of the active layer. Hayward and Clymo (1983) have shown that such a lowered water table has a negative effect on individual elongation of the stem of three species of *Sphagnum*, but collectively the growth of peat lawn increases. Earlier, however, Clymo (1973) found that increased *Sphagnum* biomass and length growth is highly correlated with a declining water table. Confounding the issue, an increase in precipitation, which is predicted as a possible result of arctic warming in the Canadian Centre for Climate Prediction and Analysis (CCC) model (Maxwell 1992), may increase water table levels. Since *Sphagnum* thrives in very moist locales such as watertracks, a higher water table could translate into increased growth of *Sphagnum* species (Moorhead & Reynolds 1993). Additionally, any change in the water table level is likely to affect *Sphagnum* species differently (Grosvernier *et al.* 1997; Hayward & Clymo 1983), and therefore could result in an altered composition of plant community and the *Sphagnum* – vascular plant competitive balance. However, changes in precipitation, evaporation, and active layer depth may nullify each other, and result in no net water table change (Gerol *et al.* 1998), and thus climate change would not affect *Sphagnum* through effects of the water table.

Light intensity is considered a key driving ecological component in many landscapes, and climate change is predicted to alter moss light environments via increased cloudiness (IPCC 2001) and/or changes in plant canopy structures (Hollister *et al.* 2005), which will affect light availability for understory species such as *Sphagnum*. Like all plants, a minimum light level is required for growth of *Sphagnum*, but the genus has a remarkable feature in which seemingly dead, old stems that are buried in a peat matrix can establish new shoots once minimum light requirements are met (Clymo & Duckett 1986). However, minimum light levels for growth activity to occur in *Sphagnum* species have not been yet determined. Haywood and Clymo (1983) concluded that higher light intensity positively correlated with higher elongation rates. Furthermore, within this generalization, they found that low light intensity on individuals of *Sphagnum* that resulted from shading by neighboring *Sphagnum* without any shading higher in the canopy, produced an increase in growth of neighbor-shaded individuals. Within the genus, this explains the community mechanism that allows individuals to regulate one another to grow in the typical mat formation (Haywood & Clymo 1983). In comparison to other mosses, *Sphagnum* species have been noted to fall on the upper end of a light saturation range from 380 to 700 μmol m^{-2}s^{-1} (Skre & Ochel 1981). At saturation however, light can become deleterious. Murray *et al.* (1993) showed that *Sphagnum* at 800 μmol m^{-2}s^{-1} is photoinhibited as indicated by a decrease in chlorophyll fluorescence, suggesting that *Sphagnum* may be inhibited if climate change resulted in an extended growing season or decreased vascular cover. Removing snow and thereby increasing light early in the growing season has been found to have similar negative response on an arctic *Sphagnum*- dominated community (S.F. Oberbauer, unpublished data).

Global annual average temperature has already increased approximately 0.6°C during the last century, and projections for the next century range from 1.4 °C to 5.8°C (IPCC 2001). Temperatures in some arctic regions may potentially increase three times the global average increase (IPCC 2001). Such increased temperatures in high latitudes may result in several direct ramifications including changes in permafrost, snow dynamics, plant phenology and physiology, evaporation and decomposition rates. Numerous circumpolar experiments have examined the response of arctic vegetation to warmer temperatures (Arft et al. 1999), as well as addressed the effects of environment variables that have been shifted due to warmer temperatures such as season length (Oberbauer et al. 2002, Starr et al. 2000, Oberbauer et al. 1998) and plant community dynamics (Walker et al. 2006).

Because temperature amplifies many other environmental variables, determining the effect that increased temperatures will have on *Sphagnum* mosses is a multidimensional problem. Harley et al. (1989) found that temperature and the photosynthetic rate of *Sphagnum* were positively correlated. Hobbie et al. (1999), however, showed through a long-term community warming experiment that non-vascular plants responded negatively to climatic warming. This negative response could have been a result of the inability of *Sphagnum* to utilize the increased net N mineralization caused by the warming (Hobbie 1996), or may be simply attributed to an increased proportion of growth in vascular vegetation in comparison to bryophyte populations, putting *Sphagnum* at a competitive disadvantage. Alternatively, Gerol et al. (1998) provide evidence that climate warming increases the growth rate of *Sphagnum*, but they make the point that no *net* accumulation of *Sphagnum* may occur because warming will also increase the rate of

peat decomposition. Lastly, warming in the Arctic may also speed up snow melt, thereby lengthening the growing season. The effect of an extended growing season on *Sphagnum*, which is conditioned to the short Arctic growing season, has yet to be investigated.

Although the seasonality of warming in the Arctic remains unclear, winter temperature increases may not affect *Sphagnum* mosses unless warming diminishes snow cover, which typical acts as an insulator against harsh atmospheric temperatures. Under snow, surface temperatures of the bryophyte understory of the Arctic have been found to remain above -10°C (S.F. Oberbauer, unpublished data). With *in vivo* measurements taken in Antarctica, Pannewitz and colleagues (2004) showed that under such insulation, photosynthetic activities may occur at low air temperatures when light levels are sufficient.

In late spring and summer, *Sphagnum* mosses and other bryophytes are exposed to a wide range of temperatures as they become snow-free (Stein *et al.* 1994). During the 3-month snow-free period, temperatures periodically plummet to subzero temperature for several hours to several days (Stein *et al.* 1994). For the period of a cold event, plants are exposed to temperatures that cause rapid freezing and thawing that could cause permanent damage. More variable weather patterns forecasted by climate change models (IPCC 2007) will continue, if not increase, the exposure of tundra plant communities to rapid freeze-thaw events. Periodic cold air outbreaks are not expected to necessarily decease in severity or frequency with warmer average temperatures (Vavrus *et al.* 2006). Furthermore, anticipated increases in daily average arctic temperatures (IPCC 2007) may

result in the loss of cold-hardening and thus decrease the ability of plants to cope with sudden cold temperature conditions, as has been observed in *Betula pubescens* (Taulavuori, K.M.J. *et al.* 2004), *Salix pulchra* (Gorsuch & Oberbauer 2002), Scots pine (Repo *et al.* 1996), and *Vaccinium myrtillus* (Taulavuori *et al.* 1997).

In this chapter, I have reviewed the potential responses of *Sphagnum* to singular changes of an array of environmental variables that may be altered directly or indirectly by global climate change. Most likely, the effect of climate change on peatland ecosystems, such as the arctic tundra, will not be driven solely by a single altered abiotic factor, but rather by diverse environmental variables, which will result in a net response that may or may not be regionally localized. Multiple environmental changes, for instance, may be responsible for the bryophyte community shift within the Finish forest floor that was observed between 1951-1995 (Mäkipää & Heikkinen 2003). Because of the need to understand the net response, research investigating the effect of climate change on *Sphagnum* that involve the manipulation of multiple climatic factors are becoming more common (Berendse *et al.* 2001, Hobbie *et al.* 1999, Jauhiainen *et al.* 1998). In the next chapter, I take just such an approach to examine the response of *Sphagnum* to temperature, light, and a common occurring weather pattern, a brief freeze-thaw event. This study investigates whether the projected warm, cloudier arctic summers with increased vascular plant shading may induce acclimation within *Sphagnum* that would result in a reduced ability to cope with short periods of subzero temperatures.

Freeze-thaw events negate the advantage of warm and shady growing environments for

Sphagnum girgensohnii

Introduction

Global warming is predicted to strongly affect the high latitudes by increasing

temperatures and altering weather patterns (Maxwell 1992, IPCC 2001). Evidence is now

overwhelming that such changes are occurring (Hinzman *et al.* 2005, Serreze *et al.* 2000).

Arctic and boreal plant communities have a comparatively short growing season that

commonly includes periodic freeze events where subzero temperatures persists from a

few hours to multiple days (Stein *et al.* 1994). More variable weather patterns forecasted

by climate change models (IPCC 2001) will continue, if not increase, exposure of tundra

plant communities to rapid freeze-thaw events. The LTER site at Toolik Lake underwent

55 FTEs during the growing seasons between 1988- 2002, 30 of which lasted more than 1

day (Shaver & Laundre 2003.) The absence of precipitation during 24 of the 55 FTEs

offered no snow cover to insulate *Sphagnum* from subzero atmospheric temperatures.

Furthermore, anticipated increases in daily average arctic temperatures (IPCC 2001) may

result in acclimation that decreases the ability of plants to cope with sudden cold

temperature conditions (Taulavuori *et al.* 2004, 1997, Repo *et al.*1996).

Studies of vascular plants have shown they often suffer irreparable tissue damage

that persists for the remainder of the growing season or longer due to these freeze-thaw

cycles (Mazur 1969). Nonvascular plant species, particularly moss in the genus

Sphagnum, compose a large proportion of the arctic communities, but research exploring

the response of nonvascular species to such freeze-thaw cycles is limited. Knowledge of

how nonvascular plants react to FTEs and mechanisms and pathways that are involved in the response to these cycles is essential to the understanding of tundra ecosystem response to low temperatures during the growing season (Chapin 1992).

The proportion of bryophytes in plant communities increases at high latitudes indicating that these plants have a competitive edge in these regions (Tenhunen *et al.* 1992). Physiological mechanisms that allow them to survive under extreme cold temperatures are one aspect of that edge. Mazur (1969) comprehensively outlined how the freezing of plants is a multiple-step process where water freezes extracellularly first, then may progress to intercellular freezing, and finally to an intracellular freezing state. The occurrence of intracellular freezing within plant tissue results in irreversible damage. Bryophytes remain in the earlier stages of freezing longer than vascular plants because of a greater ability to supercool (Dilks & Proctor 1975). Often *Sphagnum* is described as becoming 'concrete-like' when frozen (Stein *et al.* 1994). Because of the prevalence of a large quantity of extracellular water, the concrete appearance of *Sphagnum* may be a result of only extracellular water freezing, thereby avoiding frost damage. However, increased severity and/or length of a freeze may result in inter/intracellular ice formation that occurs at the on-set of frost damage.

An additional complication to freezing stress for bryophytes is that plants undergoing freezing often remain exposed to normal or even high light conditions. Some bryophytes are unaffected by high light, low temperature conditions. Lovelock *et al.* (1995) suggested that a photoinhibitory mechanism in the Antarctic moss *Grimmia antarctici* exists that allows the moss to recover from high light, subzero temperatures with little need for repair. Antarctic *Polytrichum alpestre* is another example of a

nonvascular plant that can photosynthetically recover from deep freezes, but its resilience is temperature and water dependent (Kennedy 1993). A study by Deltoro and colleagues (1999) examined how the Mediterranean moss *Leucodon sciuroides* tolerates freeze-thaw cycles by dissipative pathways. They suggest that such mechanisms may be even more important to nonvascular species in high latitudes and altitudes where low temperatures during the growing season are frequent. Even though *Sphagnum* mosses, dominant in boreal, subarctic, and arctic regions, are evidently able to survive in low temperature, high light environments, the severity and intensity of these extremes may be influencing their productivity. Murray *et al.* (1993) found that high light alone decreased *Sphagnum* mosses productivity via photoinhibition of photosynthesis. In a separate study, Balagurova *et al.* (1996) showed that *Sphagnum* mosses are highly freeze resistant, yet their resistance is variable between species and habitat. Lethal temperatures occur at about -20 °C for those species examined.

The topic of temperature acclimation of plants has received much attention, initially to understand the physiology of plants, but more recently as a potentially important component of plant interactions with the factors affected by global climate change. *Dicranum elongatum*, a model arctic species by Hicklenton & Oechel (1976), exhibited contrasting ability to temperature acclimate depending on population origin. Subarctic plants achieved temperature acclimation at a greater rate than those of arctic populations, which may suggest that favorable growing conditions optimize plasticity and physiological mechanisms that permit temperature acclimation.

Sphagnum is a significant component of arctic plant communities, accounting for approximately 27.6% of live plant biomass in the low Arctic (Hastings *et al.* 1989).

Sphagnum species are key players in this system because of their ability to maintain shallow active layers and low decomposition rates, which contribute to ecosystem storage in the Arctic. Therefore, if freeze-thaw events significantly affect the growth and productivity of *Sphagnum*, then the frequency and severity of these events may influence the ability of the Arctic to store carbon.

Here, I postulate that the physiology of *Sphagnum* depends on the temperature and light during growth, and that favorable growing conditions will result in increased ability to respond to brief subzero exposure. Further, I hypothesize that *Sphagnum* is capable of fully recovering from a mild, short-term freeze. To test these hypotheses, I conducted a growth chamber experiment on *Sphagnum girgensohni* Russow, in which I determined: (1) the rate of growth and physiological activity under different temperature and light levels; (2) the overall ability to recover from a brief freeze-thaw event; and (3) the interaction of different growth conditions with a freeze-thaw response.

Methods

Study species

Sphagnum girgensohnii Russowi. is a medium-sized, robust species that has a five-star, flat-top capitulum. Classified as a green *Sphagnum* (Plate 1a), field observations during collection revealed that exposed *S. girgensohnii* at the study site commonly exhibited a brown hue with specks of green instead of being entirely green (Plate 1b). It is positively identified via its broad, tongue-shaped stem leaves with a distinctive torn edge. Branch leaves are acute and striated, and the hardy stems are frequently reddish-brown and can exhibit a characteristic snapping sound when broken.

Collection

Seven 20 cm^2 mats of *Sphagnum* were collected along the same hillside near Toolik Lake in the northern foothills of the Brooks Range, Alaska in July of 2002 (Plate 2). *Sphagnum* was taken from non-water track, moist dwarf-shrub tundra, typical of the Alaskan Arctic (Walker *et al.* 1994), with *Eriophorum vaginatum, Betula nana, Salix pulchra*, and *Sphagnum* species dominating ground cover. Mats were place into sealable bags and were taken to Florida International University within 24 h via a cooler, where they remained at 4 °C in a dark refrigerator until preparation.

Experimental design

The experimental design consisted of Sphagnum microcosms grown for approximately 6 weeks at two temperatures (high and low, hereafter HT and LT) and two light levels (high and low, hereafter HL and LL) prior to being subjected to a 2 day freeze, followed by a 2 week recovery period. The experiment used an identical pair of environmental growth chambers set at the appropriate temperatures and the light treatments were applied equally within the growth chambers. To replicate, the experiment was repeated twice more for a total of three experimental trials. Each replicated experiment ran for 8 weeks total (55 days). All growing condition combinations (high-light/high-temperature: HLHT; high-light/low-temperature: HLLT; low light/high-temperature: LLHT; low light- low temperature: LLLT) were replicated within each experimental trial. The duplicate group within each trial received an independent, artificial freeze-thaw event so that freezing was replicated a total of six times within the experiments (Figure 1).

Sample preparation

Experimental treatments were applied to sample microcosms consisting of approximately 10-12 individual stems that were arranged in a density similar to that *in situ* in a 3 cm diameter centrifuge tube. From carefully dissected *Sphagnum* mats, individuals were separated and cut to precisely 3 cm in length including capitulum and stem, thereby harvesting the most productive portion of the individual. Any branching stems were removed to attribute any future branching to treatment effects. Samples contained individuals taken from multiple natural mats to minimize any genetic homogeneity within treatments or replications. Stems were selected to total to a mean of 0.113 g in estimated dry weight. To prepare one sample tube, 10-12 individuals were soaked in deionized water for 20 minutes, spun in a OXO Softworks Salad Spinner (model O1045409) to standardize initial water content, and weighed to establish starting biomass (Clymo 1983). A regression was performed to examine the precision of the salad centrifuge method by weighing a subsample of spun plants, drying the samples for 48 hours at 70 °C, and recording the dry weight. The method was deemed acceptable for standardizing water content (R^2= 0.92, n=32, Figure 2). Digital images were taken to document overall qualitative condition and color of each moss sample. Canadian peat moss (Scotts®) served as the substrate in the tubes below the 3 cm length plants. The substrate was heated to 70 °C for 48 h to destroy any potential competing foreign species. Sixteen sample tubes were prepared for each replicate of the experiment.

Growth chamber conditions

A pair of EGC 15 growth chambers (Environmental Growth Chambers, Chagrin Falls, OH) were used to provide the treatment conditions. One chamber was set at 12 °C for 18 h out of a 24 h period and 10 °C for the remaining 6 h. The substrate portion of the sample tubes was submerged in an icebath in the 12 °C chamber to simulate a shallow active layer. The second chamber was set at 18 °C for 18 h and 14°C for 6 h. In both chambers, light levels were maintained at ~475 μmol m^{-2}s^{-1} photosynthetically active radiation for 18 h out of a 24 h period with high output fluorescent bulbs. Photoperiod was maintained at 24 h in both chambers by running one 100 W incandescent bulb continuously. Low light conditions (~325 μmol m^{-2}s^{-1}) were created by placing 50% shade cloth caps on four of the eight sample tubes in every chamber. All samples were saturated with deionized water daily; high-light samples received additional watering as needed to compensate for potential higher evaporation rates. Temperature and light levels in the chambers were checked daily to ensure standardized levels throughout the experiment using a precision thermocouple multimeter (Tenma, Springboro, OH) and a LI-180 light meter (LI-COR, Inc., Lincoln, NE), respectively.

Sampling methods

Gross primary productivity (GPP), overall photochemical quantum yield (YIELD), and dark-adapted chlorophyll fluorescence (F_v/F_m) were measured for each sample tube once a week for 5 weeks during the pre-treatment/acclimation period (Days 8, 15, 22, 29, and 36), and two times following the subzero treatment/ recovery period: 5 days after the freeze and 2 weeks after the freeze (Days 46 and 55). For those samples

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that underwent the freeze event (Day 40), measurements were also made at initiation and conclusion of the event at $6\,^{\circ}C$ (Days 39 and 41). Gross primary productivity was calculated by combining net primary productivity with respiration rates. Net photosynthesis was measured in a closed-system chamber whereby each sample tube was placed in a 1 L cuvette attached to a LI-6200 Portable Photosynthesis System (LI-COR, Inc., Lincoln, NE). Respiration rates were determined by taking a second measurement of each sample with a dark cloth over the cuvette. Both YIELD and F_v/F_m of all samples were measured with an OS5-SP Fluorometer (OptiSciences, Inc. Tyngsboro, MA). Dark conditions needed for measurements were achieved by briefly turning chamber lights off. All pre/post-freeze measurements were taken at 18 °C, and moss was water saturated < 1 h prior to all measurements.

Growth was evaluated through four components: biomass accumulation via net dry weight (NDW), net elongation of the main branch (NEMB), frequency of branching measured by summing the total number of new branches (NB), and the total new branch length per microcosm (NBL). All growth measurements were taken at the initiation of the experiment and 8 weeks later (Day 56), at its conclusion.

Treatment: freeze-thaw event

After all samples were watered, one sample out of the four growing in each light/temperature growing condition combination was subjected to an artificial freeze-thaw event. A second, independent freeze-event was performed on the other half of the experimental samples of the trial approximately three days after the first event. The 36-hour, freeze-thaw event began after the completion of the fifth week of pretreatment

measurements. Experimental sample tubes were placed in a cooler of ice, and the cooler was placed into a 6 °C chamber (EGC 15, Environmental Growth Chambers, Chagrin Falls, OH). Each sample was equipped with a fine, 15 gauge copper constant thermocouple attached to a Campbell CR10X datalogger (Campbell Scientific, Login, UT). After 12 h at 6 °C, physiological measurements were taken. After the samples were covered with Parafilm® (American National Can, Chicago, IL) to prevent water addition from the freezer or ice, the samples were placed at 0 °C for 12 h by covering tubes with ice and placed in a 2 °C refrigerator for 12 h. At this time, light levels were reduced to zero for control sample tubes with no change in temperature conditions to prevent a difference in total potential growing time between control and experimental plants. To continue the freezing process, samples were moved to a -20 °C freezer for 12 h where the temperature of the samples reached a low of –12 °C (Figure 3). Because literature that estimates the lethal cold temperature for *S. girgensohnii* does not exist, the minimum temperature chosen was based on the lethal temperature of the least freeze resistant *Sphagnum* moss yet reported, *S. magellanicum*, which is –16.6 °C (Balagurova *et al.* 1996). Temperature was maintained at all times at least 4 °C above this lethal temperature. All six FTE averaged -5.04 ± 0.13, which is similar to the average temperature of FTEs that occurred at Toolik Lake, AK, on August 30, 1988 (-6.9°C); September 12, 1991 (-5.2°C); September 1, 1993(-4.5°C); June 1, 1997 (-4.2°C); May 28, 2002 (-6.5°C) (Shaver & Laundre 2003.). After the 12 h subzero period, sample tubes were returned to control conditions in the reverse order of temperatures and at the same rate they were taken to reach the climax of the FTE.

Statistical methodology

All data was analyzed using the statistical program SAS (SAS v 9.1, SAS Institute, Cary, NC). Prior to analysis, normality and heterogeneity of variances of all data were verified. Growth data were examined using a split-plot design to minimize any within chamber effects. Physiology data collected to compare light-temperature interactions prior to any freeze event were analyzed using a split-plot design with repeated measures. Lastly, a split-split plot design was utilized to examine the response of the freeze-thaw event. For the purposes of this study as a result of the small sample sizes, I consider $p < 0.1$ to be statistically significant.

Results

Light-temperature growing environments

Overall, light treatments exhibited a significant influence on the growth of the control samples: net dry weight ($p = 0.0092$), net elongation of main branch ($p = 0.0240$), number of new branches ($p = 0.0848$), new branch length ($p = 0.0532$) (Table 1). The number of new branches was found to vary strongly also among temperature environments ($p = 0.0015$), which lead to yielding a significantly different response among the light-temperature regimes ($p = 0.0488$) (Table 1). Over the 8-week experimental period, net dry weight (NDW) was greatest in the low light environments ($p = 0.018$) with the highest NDW found in the LLHT conditions ($x = 0.215 \pm 0.122$ g, Figure 4a, Plate 4). The change in the main branch length or net elongation of main branch (NEMB) was also greatest in low light conditions ($p = 0.024$). Individuals from the LLLT environment had the largest mean length increase of 0.37 ± 0.07 cm compared

to the other three growing environments, where means ranged from 0.16 ± 0.06 (LLHT) to 0.22 ± 0.07 cm (HLLT) (Figure 4b). New growth in the form of number of branches (NB) (Figure 4c) and length of those new branches (NBL) (Figure 4d) were largest in the LLHT growth environment with the mean number of new branches equaling 25.2 ± 4.7 and a mean total branch length per sample of 35.60 ± 10.61 cm (Plate 5).

Weekly physiology measurements prior to freeze treatment revealed that gross primary productivity (GPP) was generally higher for samples grown in high-temperature environments regardless of light (Figure 5a). No difference in GPP was found within the first week, but values in the second week showed a significant increase for high-temperature samples over GPP of the low-temperature samples (Figure 5a), which produced a statistically significantly temporal difference between temperature regimes (Table 2). By week three however, all samples had established similar GPP rates with the highest GPP occurring in the low-light, high-temperature environment (Figure 5a). All treatments showed a reduction at week 5 prior to freeze treatment. Because samples were growing over the period, these values potentially reflect both changes in tissue photosynthetic capacity as well as changes in the amount of photosynthetic tissue.

The average photochemical quantum yield (YIELD) during the 5 week period pre-freeze treatment ranged from 0.446 ± 0.011 to 0.562 ± 0.026, and showed a similar trend as GPP with LLHT samples showing the highest YIELD values (Figure 5b). However differences in YIELD between light-temperature environments were not statistically significant (Table 2). Similar to the temporal GPP trend, low light levels produced higher YIELD during weeks 2 and 3 (Figure 5b), which accounts for the significant light*week variation (Table 2).

Results of dark-adapted chlorophyll fluorescence (F_v/F_m) exhibited the same tendency as GPP and YIELD during the five-week pre-freeze period, where samples had the highest mean F_v/F_m in the LLHT environment (x = 0.652) and the lowest mean F_v/F_m were from the HLLT environment (x = 0.585, Figure 5c). Throughout the pre-freeze period, the mean F_v/F_m in the LLHT environment each week remained almost constant and consistently above the mean F_v/F_m for other environments, with the exception of LLLT that peaked during week 3 (Figure 5c). Overall, the effects of temperature (p = 0.004) and the effects of light (p = 0.095) on F_v/F_m differed during the growing period.

Changes in physiological activity of Sphagnum girgensohnii during freeze treatment

Immediately prior to freezing, physiological measurements were taken on experimental samples of *S. girgensohnii* once their temperatures had attained 6 °C for a minimum of 4 h, and samples were measured again at the conclusion of the freeze after samples had readjusted to 6 °C for 4 h. The 6 °C conditions at the commencement of the freeze depressed GPP for experimental samples, down 36.2% from the same samples measured the previous day at 18 °C (Figure 6a). Also affected by the initial 12 °C decrease, YIELD was reduced by 24.2% (Figure 6b). However, F_v/F_m was only slightly reduced, 8.9% (Figure 6c).

Physiological measurements taken at 6 °C at the conclusion of the freeze event indicated a significant reduction in GPP for samples grown in all light-temperature environments (p < 0.001) (Table 3). Overall, *S. girgensohnii* underwent a 44.3% average decrease in GPP from measurements taken at 6 °C over all FTE samples, with HLLT samples showing the greatest decrease on average, 49.1% (Figure 6a). The freeze reduce

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the YIELD of all plants (p = 0.027), but the FTE impact was shown to have reduced the YIELD of LL regimes more (p = 0.092) (Table 3). Specifically, changes in YIELD differed among the treatments where LLHT plants decreased the most, falling by an average of 35.1% (Figure 6b). Samples from LLHT also exhibited a 15.1% decrease in F_v/F_m ratio, but variations did not show significance. (Figure 6c, Table 3).

Rate of physiological recovery from a freeze-thaw event

The freeze-thaw event caused an observable loss of chlorophyll in the newest tissue (Plate 6), suggesting that the FTE was particularly damaging to the photosynthetic apparatus of young tissue. Gross primary production of samples that underwent freezing remained depressed over the 2-week recovery period: Day 36 verse Day 46 (p =0.021) (Table 4); Day 36 verse Day 55 (p < 0.001) (Table 5) (Figure 7a). At the conclusion of the 8-week growth period (Day 55), control samples all showed greater GPP when compared to their GPP during week 5. Plants grown under high temperature that underwent a FTE also showed an increase in GPP when compared to GPP measurements collected week 5, however, those increases were small in comparison to the increased GPP of control samples grown in the high temperature treatments. The GPP of low temperature treatment plants was reduced after the FTE compared to levels measured prior to the FTE, as well as in comparison to GPP of low temperature controls measured during week 8. In response to a FTE, GPP of samples in HLLT environments declined by 28.1% and those in LLLT declined 22.7% compared to values from week 5. Compared to control samples at week 8, the percent reduction in GPP due to the FTE was even larger: HLHT (13.3%); HLLT (34.2%); LLHT (44.4%); LLLT (50.2%). A GPP comparison of

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the difference between Day 36 & 46 and Day 36 & 55 reveal a slight significant variation with Day 55 reflecting small physiological changes during this recovery period. (Table 6, Figure 7a).

During the two weeks following the freeze, changes in YIELD values from before the freeze to after in both control and freeze-treated samples were minimal (Figure 7b). However, a comparison of YIELD changes between Day 36 and Day 46 shows a significant difference between frozen and control plants ($p = 0.021$). Those plants growing in HT environments actually displayed a small increase in YIELD six days into the recovery period. Fourteen days post-freeze, slight reductions in YIELD were found in the samples grown in LLHT (9.1%) and LLLT (6.0%) conditions when compared to control samples. However, collection of fluorescence data for both YIELD and F_v/F_m became more difficult in FTE samples for which no appreciable F_0 signal was found in initial measurements, and often multiple attempts were necessary to obtain any reading.

Likewise, differences observed in F_v/F_m from before the freeze compared to after the freeze in both control and FTE samples were significant six days after the FTE with frozen samples having an increased F_v/F_m when recovering in a HT environment (Table 4, Figure 7c). Values of F_v/F_m taken 14 days after the freeze indicate that differences between FTE and control plants may have disappeared during this recovery period. (Table 5, Table 6, Figure 7c).

Growth impacted from a freeze-thaw event

Growth in all four categories was reduced from exposure to a FTE: NDW ($p = 0.015$); NEMB ($p = 0.035$); NB ($p = 0.009$); NBL ($p < 0.001$) (Table 7, Figure 8a). The

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freeze-thaw event caused an observable loss of chlorophyll in the newest tissue (Plate 6). Dry weight accumulation measurements (NDW) taken after the recovery period revealed that the samples grown under low light treatments were quantitatively more negatively impacted by the FTE than those grown in high light environments (Figure 8a). The traditional field assessment for *Sphagnum* growth, NEMB, lacked any interaction between light environments and the response to the freeze-thaw event. In contrast, the main effect of temperature was significant for NEMB in response to the FTE; plants grown in the colder treatments fared the worst ($p = 0.007$).

Without encountering a FTE, the number of new branches of *Sphagnum* (NB) and the length of those branches (NBL) were significantly higher in HT environments than LT ones ($p = 0.042$, $p = 0.057$, respectively) (Table 8). However, this advantageous growth found in HT environments was lost after the FTE (Table 8). Comparing each light-temperature environment against one another, three cases of such a loss in growth, which was produced in more ideal pre-freeze conditions, were observed (Table 9). The shading advantage plants gained to produce a significantly high number of branches (NB) and new branch length (NBL) in LLHT environment over the HLHT environment was lost (Table 9). Likewise, shading no longer posed as an advantage to when comparing those plants that grew at low temperatures; the main branch (MBEL) of LLLT plants were significant longer than HLLT plants when no FTE was encountered ($p = 0.021$), but the difference was not significant in those samples which experienced a FTE ($p = 0.279$) (Table 9.) The remaining instances of growth occurring in one light-temperature environment being not significantly different from that of another environment before

FTE and then shifting to an insignificant variation or vice versa is most likely due to the large standard error among the samples growing the better performing environments.

Discussion

Preference for a low light-high temperature environment

Sphagnum girgensohnii grown in LLHT condition had the greatest overall growth including NDW, NB and NBL and productivity in terms of GPP, YIELD and F_v/F_m. Low light conditions in this experiment simulate partially shaded *in situ* environments, which have been found to encourage *Sphagnum* growth and productivity. Foremost, partial shading has been argued to be a requirement for the peat mat formation. The ability of *Sphagnum* individuals to grow as uniform mats has been attributed to individual ramets increasing growth rates upon shading by nearest *Sphagnum* neighbors, often referred to as self shading (Malmer *et al.* 1994).

The average photochemical quantum yield (YIELD) over the 5 week pre-freeze treatment showed *Sphagnum* in low light environments performing better. Visual comparison of HL and LL samples showed the latter having transition from an *in situ* brown color to predominantly green suggesting as the YIELD has that the quantity of chlorophyll increased under the low light environment to a measurable level in only a few weeks.

Higher solar irradiance *in situ* has been suggested to result in photoinhibition in the genus *Sphagnum* (Murray *et al.* 1989). In environmental growth chamber experiment, Murray *et al.* (1993) found that high light (800 μmol photons $m^{-2}s^{-1}$) caused

photoinhibition of photosynthesis. Although our high light treatment was almost 50% less than this known photoinhibitory level, *S. girgensohnii* grown under our higher chamber light (450 umol photons $m^{-2}s^{-1}$) may have been experiencing partial light saturation at the time physiological measurements were taken for all samples at high light conditions.

Low light resulting in shading of vascular plants has also been seen to increase growth in *Sphagnum,* as in the process of etiolation. Furthermore, low light conditions caused by vascular plants have been show to reduce evapotranspiration allowing *Sphagnum* to retain water and soluble nutrients within and upon the hyaline cells (Plate 7) that is crucial to productivity (Heijmans *et al.* 2001). Lower evapotranspiration rates were likely an important contributing factor to the high growth and productivity of LL plants as a result of shading, despite daily water saturation of all samples.

Warmer climate preference

Statistically, low light environments produced *S. girenesohnii* that had higher rates of NEMB, which points to etiolation. However, the LLLT environment was responsible for the difference in NEMB rates between low and high light environments (Figure 4B). Under LLLT conditions, mosses shifted from growing via new shoots towards main stem elongation, suggesting that temperature may cue a change in hormonal control of growth. Field methodology that uses stem elongation only to evaluate growth, such as the crank-wire method (Roy *et al.* 2000), would potentially allow this temperature driven growth shift to go undetected. However, it is unclear from my results whether differences in NDW can solely be attributed to stem elongation

(NEMB) and/or branching (NB & NBL) or if changes in leaf morphology and leaf mass may be contributing to NDW. The ecological implications of such a shift in growth form in response to temperature is that the spread of peat mats via new shoot regeneration is more difficult at lower temperatures, and thus, potentially at higher elevations, where it is typically colder during the growing season. This conclusion is circumstantially supported by the decreased prevalence of *Sphagnum* from boreal to high arctic regions.

Regeneration via branching

Unexpectedly, all simulated growing conditions resulted in branching, complete with capitula, at a higher rate than observed in the field. This finding may be a result of chamber conditions differing in some unique way from ideal growing conditions. Clymo & Duckett (1986) argue that innovations responsible for branching typically remain dormant in the top few centimeters of green *Sphagnum* due to some apical dominance mechanism, which conflicts with these findings. The storage time between *in situ* harvest and initiation of the experiments caused the capitula of most plants to lose greenness. Although *Sphagnum* plants that branched had green capitula at the time of harvest, their stressed state at the start of the experiment, as indicated by homogenous brownness, may have released any apical dominance. Such a regeneration alternative might indicate that branching can occur following a winter when chlorophyll is in lower concentrations in capitula compared to the peak of a growing season.

Alternatively, the high rate of branching in our findings may be in response to greater light availability below the capitula in my microcosm compared to that found within mats *in situ*, irrespective of any change in apical dominance. Such a branching

response to decreased shading of moss stems could serve as a recovery mechanism of a disturbed mat.

Investigations examining above and below biomass growth often occur late in the arctic growing season. This method may preclude detection of below surface branching in *Sphagnum* because one or more freeze event(s) may have occurred during the season, which could eliminate any young, tender offshoots. Harvesting of materials to transport for this growth chamber experiment in summer 2002 took place after two strong summer snowstorms and freeze thaw events. Some minimal branching was observed and removed as the natural mats were disassembled for preparation for this experiment. More branching may have occurred and then died back within the season prior to harvest.

Changes in physiological activity in Sphagnum during a freeze-thaw event

Reduction in temperature to 6 °C at the beginning of the FTE correlated with lower physiological activity, as expected. Low GPP at this reduced temperature indicated that *Sphagnum* is highly sensitive to temperature changes. As indicated by YIELD, the efficiency of photosynthetic electron transfer declined quickly, although the quantum yield indicated by F_v/F_m remained high.

The reduced GPP collected at the end of the FTE at 6 °C showed that plants from all light-temperature environments were impacted, and at the time of post FTE measurements, partial intracellular and intercellular freezing may have still persisted, contributing to depressed physiological activity. With the greatest reduction in GPP, *S. girgensohnii* grown in HLLT also had the highest number of tender new branches except for LLHT plants (Figure 4c). Given the small size of new branches in the HLLT category

(Figure 4d), they may have been too young to survive the FTE and their subsurface destruction may be the source of the substantial loss of GPP immediately following the FTE.

In the LLHT environment, where plants grew best, both YIELD and F_v/F_m ratio declined strongly in response to the FTE. Even if the FTE caused substantial shoot damage, the large volume of moss below the surface in these samples may have been able to mask the damage in terms of GPP. Fluorescence measurements indicate that at least on the surface the LLHT moss lost some photosynthetic capability and exhibited stress following the FTE.

Sphagnum growth at low-temperature: physiologically vulnerable to freeze-thaw events

After the FTE, *Sphagnum* samples under all treatments displayed reduced GPP that remained low until the conclusion of the experiment. Those plants that had not undergone a FTE showed increased GPP over values taken before FTE, indicating that nutrient limitation or a decline in the quality of the growing environment did not account for the decrease in GPP found in the FTE treated samples. High temperature environments, regardless of light levels, produced samples with higher GPP after the FTE than before, but still lower than their control counterparts. High temperatures may have promoted increased vegetative biomass so that sufficient sample remained physiologically intact following the FTE to respond positively during the recovery period. However, samples growing well prior to the FTE may show a large effect from the FTE, as seen by comparing the treatment effect to measurements taken prior to the FTE (Figure 8).

Although not statistically shown, freeze-treated *Sphagnum* grown at low temperature seemed to display a deteriorated state after freezing. The cool temperatures following the FTE may have prolonged the negative effects of FTE whereby intracellular and intercellular freezing may have continued for a more extended period and exposure to growth light conditions during the longer thawing period may have caused photosynthetic damage (Murray *et al.* 1993). This suggestion is supported by the finding of lower F_v/F_m, a sensitive measure of stress, only for low temperature samples. Furthermore, following thawing low temperatures may have limited photosynthetic activity and thereby the ability of the plants to repair photosynthetic damage.

Freeze-thaw event: modes of growth and growing conditions yield differing responses

The effect of a FTE on the four different measurements of *Sphagnum* growth add further evidence that the environment may trigger *Sphagnum* plants to shift from elongation to branching or vice versa. Examination of NDW changes alone do not reveal this process, however, but does support my hypothesis that *Sphagnum* grown in a favorable growing environment, such as the low light environment, incurs more damage from a FTE. Losing the least NDW from the FTE, *Sphagnum* from the HLLT may have had the least photosynthetic damage as a result of low growth and photosynthetic activity in the five weeks prior to the FTE, as indicated by the GPP data. Exposure to the stress of high light may have preconditioned the plants for the stress of freezing and thawing. Such a characteristic would be especially advantageous for *Sphagnum* species during early spring, as it would ensure quick recover from fluctuating temperatures at the beginning of the growing season. Once temperatures warmed, the density of capitula

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could quickly return filling out the tundra mat. However, due to the severity of this experiement FTE, this cannot be verified, since different light-temperature regimes seemed to do little in preparing the most vunenable new shoots.

Sphagnum individuals grown under low temperature were found to have lost more of their NEMB growth from the FTE. Phenological and physiological damage from naturally occurring FTE have been observed for arctic vascular plants (Gorsuch & Oberbauer 2002). However, damage to Sphagnum mosses and changes in mat heights have been more difficult to observe, given the naturally occurring changes in hydrology during a FTE and the limitations in measuring Sphagnum growth. I believe that these are the first data that indicate that Sphagnum growing in the cold tundra may be negatively impacted by severe FTE, including loss of main stem elongation that halts further mat advancement. Thus, FTEs may not give Sphagnum species any advantage over vascular plants as all plants, vascular and nonvascular species alike, need a recovery period from such an event.

Although the range of measured NEMB may appear small (0.07- 0.37 cm), it is comparable to field observations of growth over an 8-week period, but with much better precision. The crank-wire method is typically used to measure NEMB of Sphagnum where it is challenging to measure to better than 0.3 cm. In the laboratory, measurements were collected with more precision (0.1 cm) and accuracy due to the dissection of the mat.

Damage due to the FTE was most visible when growth was evaluated via NB and NBL. Such a response shows that Sphagnum grown in any environment containing an occasional FTE may lose branch growth. Therefore FTE may operate as a factor affecting

Sphagnum growth and the occurrence of FTE may be positively correlated to *Sphagnum* mat advancement. *Sphagnum* grown in low temperatures yielded damage to both NB and NBL, allocating future growth to elongation. Furthermore, LLHT plants displayed some loss of new branches and new branch length after a FTE, and this research revealed that a severe FTE may cause *Sphagnum* to lose any advantages a warmer, shadier environment may have offer. During the recovery period, LLHT may have responded to this loss in branches by allocating surviving resources to grow to main stem elongation. Hence, the 2 week recovery period may be responsible for producing LLHT individuals with longer main branches than plants grown in the LLHT continuously for 8 weeks without any damaging FTE. If this is the case, a warmer, shadier environment in the Arctic with the occasional FTE, may push *Sphagnum* to continue to grow primarily via their main branches, thereby continuing mat advancement and potentially influencing the vascular community.

Warmer Climate: Sphagnum Loses Ecosystem Engineering Ability

Sphagnum has been classified as a potential ecosystem engineer, modifying the environment for its own persistence (Svensson 1995, Van Breemen 1995). When grown with *Sphagnum*, vascular plants allocate primarily to vertical growth to prevent engulfment by the upwardly advancing *Sphagnum* mat (Malmer *et al.* 1994). Prior to my findings, *Sphagnum* was assumed to chiefly utilize one mode of growth typically measured with the crank-wire method. Interactions between abiotic factors, vascular plant growth, and the growth strategy of *Sphagnum* have yet to be described. In Figure 9, I present a model that summarizes these interactions.

A cold Arctic with cool summers preserves *Sphagnum* and its engineering role in the ecosystem. With minimal vascular vegetation in an environment of HLLT, *Sphagnum* grows slowly with minimal branching causing an advancing mat, which results in vertical vascular growth (Svennson 1995). This pressure coupled with cold temperatures and a short growing season results in vascular plant growth allocated to height but not density for shrubs such as *Betula* and *Salix*, thereby maintaining a high light environment for *Sphagnum* species. In environments with more favorable conditions such as increased nutrient availability, shrub leaf density may increase in addition to vertical growth, decreasing the light environment for *Sphagnum* below. Such a shift may be short term, however, because the growth strategies of *Sphagnum* may act as a negative feedback. The colder, lower light environment in this experiment triggered increased elongation, and therefore a quick increase in mat height, which results in an increased need for vertical vascular plant growth. With resources allocated to vertical growth, vegetative cover is minimized and higher light conditions are returned for the *Sphagnum* understory.

Under warming climate conditions, the stability of this peat-dominated system may erode. A warmer, high light environment, such as HLHT condition in this experiment, would increase *Sphagnum* growth through elongation and some branching. Growth would probably be modest as it was in our experiment. Many vascular species are expected to positively respond to increases in summer temperatures and longer growing seasons via earlier snowmelt (Walker *et al.* 2006). As the *Sphagnum* mat advances and vascular competition for nutrient availability increases, small evergreen and forb species may be initially negatively affected. Deciduous shrubs such as *Salix* and *Betula* species could increase in both height and leaf density, resulting in a potential

stable and preferred low light environment for the *Sphagnum* below. Our data showed that the LLHT environment resulted in high growth largely attributed to a high level of below surface branching. With *Sphagnum* allocating growth to branching and less to elongation, the *Sphagnum* mat may slow its advance and thereby (1) halt any negative impact it may have on small evergreens and forbs and (2) remove pressure on deciduous shrubs to grow vertically, which allows vascular cover to maintain a low light environment for *Sphagnum*.

Sphagnum and permafrost

Under warming microclimate conditions in the Arctic, woody shrubs such as *Betula* and *Salix* species positively respond with increase vegetative cover (Kullman 2002), which would produce a favorable lower light environment for *S. girgensohnii*, a species commonly found in almost full light throughout the Brooks Range, Alaska. The preferred low light coupled with warmer temperatures may increase nonvascular growth, thereby mitigating active layer expansion with the nonvascular mat's insulating characteristics. In a multi-dimensional dynamic climate, however, *Sphagnum* may support the growth of woody shrubs to its own detriment due to shifts in the allocation of growth. Low light with higher temperature resulted in increased growth via branching that collectively increases mat density, perhaps a short-term mechanism against belowground temperature increase and indirect vascular competition. Such a mechanism may mitigate permafrost decline for an occasional warmer arctic growing season, but coupled with a warming trend, may destabilize the permafrost and existing plant community over a series of consecutive warmer seasons for several reasons.

(1) A denser mat may serve to isolate atmospheric temperatures from the upper areas of permafrost over a season, but the lack of vertical *Sphagnum* growth over many years may allow collective warming belowground as the distance between permafrost and atmosphere is reduced. Heat could be transferred across this distance via extracellular water found on *Sphagnum* species.

(2) An increase in mat density allows the retention of extracellular water by reducing evapotransipartion. Waddington *et al.* (2001) found that higher temperature and soil moisture resulted in increased production rates in peat. This acting alone would facilitate further growth *Sphagnum* and insulating below. However, with permafrost levels already falling in warmer microclimates (Jorgenson *et al.* 2006), dropping water tables are expected to follow (Woo & Young 2006). In fens primarily, water retained initially in the dense *Sphagnum* mats may be lost to the competing vascular plants, giving them a successional advantage.

(3) A denser *Sphagnum* mat may be more hospitable for germination of some vascular seedlings. Many tightly woven *Sphagnum* individuals could serve to supply a vascular seed with amply water and nutrients and cradle it closer to the surface than a typical mat. If such parameters are improved for a particular vascular form, then it may have a short-term competitive advantage over the rest of the plant community.

(4) Vascular plants grown with *Sphagnum* have exhibited pressure to produce vertical growth. Changes in mat density may change the competition that occurs within the vascular community.

Freeze-thaw events as an ecosystem influence

Freeze-thaw events interrupt the growth and physiology of *Sphagnum*. *Sphagnum* growing in optimal growing climates may be more likely to be affected by such events, not because of their overall lack of cold temperature acclimation, but rather because of greater loss of new biomass. Continuous loss of new tissue due to frequent FTE may reduce the likelihood of branching, which may influence mat regeneration, an important issue in restoration of northern bog ecosystems.

The lack of FTE in a warmer climate may result in a stalling of *Sphagnum* mat advancement as a result of increased branching. Such a combination of a continuously warmer growing season without periodic "cold snaps" may reduce the competitive pressure for vascular plants to grow vertically. In contrast, the presence of FTE in a warmer Arctic may provide a mechanism in which *Sphagnum* species continue to grow vertically because the occasional FTE damage tender new branches. Thus the advancement of the *Sphagnum* mat may continue under a warmer climate given the occurrence of FTEs.

Table 1. Analysis of variance of growth response to light and temperature: net dry weight (NDW); net elongation of main branch (NEMB); total number of new branches per sample (NB); total new branch net elongation per sample (NBL). D.F. = 1. Bolded values are statistically significant at p < 0.10. Plants never incurred a FTE.

Source of Variation	NDW		NEMB		NB		NBL	
	F	p	F	p	F	p	F	P
Light	7.52	**0.009**	5.73	**0.024**	3.13	**0.085**	4.04	**0.053**
Temperature	0.32	0.577	0.17	0.696	12.97	**0.002**	9.55	**0.027**
Light*Temp	0.08	0.777	2.08	0.161	4.14	**0.049**	2.10	0.157

Table 2. Analysis of variance of physiological response to light and temperature during 5 weeks of growth: gross primary productivity (GPP); overall photochemical quantum yield (YIELD); dark-adapted chlorophyll fluorescence (Fv/Fm).

Source of Variation	GPP		YIELD		Fv/Fm	
	F	P	F	p	F	p
Light	2.61	0.152	7.08	0.113	8.38	**0.006**
Temperature	4.03	0.130	1.69	0.260	2.19	0.214
Light*Temp	0.46	0.520	0.11	0.737	0.49	0.485
Light*Week	1.49	0.222	3.48	**0.015**	2.12	**0.095**
Temp*Week	6.97	**<0.001**	1.91	0.126	4.47	**0.004**
Light*Temp* Week	1.47	0.244	0.31	0.9015	0.30	0.907

Table 3. Analysis of variance of physiological response between Day 39 and Day 41 under light, temperature and FTE differences measured at 6°C: gross primary productivity (GPP); overall photochemical quantum yield (YIELD); dark-adapted chlorophyll fluorescence (F_v/F_m). FTE occurred on Day 40.

Source of Variation	GPP		YIELD		F_v/F_m	
	F	P	F	P	F	p
Light	0.30	0.582	0.00	0.973	0.38	0.546
Temperature	0.02	0.904	0.61	0.473	0.01	0.938
Freeze	41.01	**<0.001**	5.71	**0.027**	0.93	0.347
Light*Temp	0.00	0.953	0.45	0.510	0.03	0.862
Light*Freeze	0.08	0.778	3.14	**0.092**	2.07	0.166
Temp*Freeze	0.01	0.931	0.47	0.499	0.72	0.408
Light*Temp *Freeze	0.04	0.956	0.24	0.786	0.08	0.924

Table 4. Analysis of variance of physiological difference between Day 36 and Day 46: gross primary productivity (GPP); overall photochemical quantum yield (YIELD); dark-adapted chlorophyll fluorescence (F_v/F_m). FTE occurred on Day 40.

Source of Variation	GPP		YIELD		Fv/Fm	
	F	p	F	P	F	p
Light	0.61	0.440	5.03	**0.031**	3.35	**0.097**
Temperature	0.56	0.532	0.72	0.484	0.03	0.877
Freeze	5.84	**0.021**	3.25	**0.080**	12.95	**0.005**
Light*Temp	0.25	0.617	0.33	0.570	0.01	0.920
Light*Freeze	0.07	0.788	0.36	0.550	2.19	0.169
Temp*Freeze	0.05	0.827	0.21	0.653	6.15	**0.032**
Light*Temp *Freeze	0.19	0.666	0.67	0.418	13.56	**0.004**

Table 5. Analysis of variance of physiological difference between Day 36 and Day 55: gross primary productivity (GPP); overall photochemical quantum yield (YIELD); dark-adapted chlorophyll fluorescence (F_v/F_m). FTE occurred on Day 40.

	GPP		YIELD		Fv/Fm	
Source of Variation	F	p	F	p	F	p
Light	1.83	0.185	0.10	0.757	0.14	0.710
Temperature	0.04	0.866	0.03	0.876	0.03	0.874
Freeze	14.58	**>0.001**	0.04	0.851	0.27	0.605
Light*Temp	0.13	0.716	0.41	0.524	0.08	0.775
Light*Freeze	1.41	0.242	0.04	0.836	0.00	0.950
Temp*Freeze	0.07	0.794	0.00	0.996	0.00	0.978
Light*Temp *Freeze	0.57	0.454	0.09	0.761	0.25	0.623

Table 6. Analysis of variance of physiological difference between Day 46 and Day 55: gross primary productivity (GPP); overall photochemical quantum yield (YIELD); dark-adapted chlorophyll fluorescence (F_v/F_m). FTE occurred on Day 40.

	GPP		YIELD		Fv/Fm	
Source of Variation	F	p	F	p	F	p
Light	0.23	0.634	0.28	0.603	2.65	0.120
Temperature	0.01	0.915	3.41	0.206	0.01	0.943
Freeze	2.56	0.100	0.93	0.341	1.17	0.292
Light*Temp	0.21	0.647	0.06	0.802	1.49	0.238
Light*Freeze	0.04	0.965	0.00	0.979	0.06	0.810
Temp*Freeze	0.96	0.392	0.93	0.342	1.72	0.205
Light*Temp *Freeze	0.30	0.825	0.17	0.680	0.23	0.634

Table 7. Analysis of variance of growth response to a freeze-thaw event and interactions with light and temperature: net dry weight (NDW); net elongation of main branch (NEMB); total number of new branches per sample (NB); total new branch net elongation per sample (NBL).

Source of Variation	NDW		NEMB		NB		NBL	
	F	p	F	p	F	p	F	p
Freeze	6.54	**0.015**	4.78	**0.035**	7.60	**0.009**	17.81	**<0.001**
Light*Freeze	1.16	0.288	0.03	0.873	0.43	0.514	1.42	0.243
Temp*Freeze	0.28	0.599	8.31	**0.007**	0.86	0.359	0.04	0.858
Light*Temp *Freeze	0.02	0.891	1.32	0.258	1.37	0.250	1.76	0.154

Table 8. Analysis of variance- Difference of least squares means of growth response comparison among light and temperature environments with and without a FTE: net dry weight (NDW); net elongation of main branch (NEMB); total number of new branches per sample (NB); total new branch net elongation per sample (NBL). Comparisons of light-temperature environments that display a departure in significance due to a FTE are underlined.

	NDW		NEMB		NB		NBL	
FTE	N	Y	N	Y	N	Y	N	Y
Growing Environments	p	p	p	p	p	p	p	p
HL vs LL	0.6699	0.3375	0.3799	0.2829	0.3328	0.2510	0.1380	0.1885
HT vs LT	0.0184	0.0111	0.1115	0.1348	0.0420	0.3664	0.0570	0.2941

Table 9. Analysis of variance- Difference of least squares means of growth response comparison between light-temperature environments with and without a FTE: net dry weight (NDW); net elongation of main branch (NEMB); total number of new branches per sample (NB); total new branch net elongation per sample (NBL). Comparisons of light-temperature environments that display a departure in significance due to a FTE are underlined.

	NDW		NEMB		NB		NBL	
FTE	N	Y	N	Y	N	Y	N	Y
Growing Environments	p	p	p	p	p	p	p	P
HLHT vs HLLT	0.2219	0.6691	0.7474	0.3647	0.6471	0.3764	0.6721	0.3124
HLHT vs LLHT	0.4022	0.0158	0.8575	0.2846	0.0023	0.2567	0.0124	0.2133
HLHT vs LLLT	0.1748	0.6043	0.2066	0.9340	0.8980	0.4117	0.7200	0.3755
HLLT vs LLHT	0.0628	0.0461	0.6922	0.0759	0.0809	0.1676	0.0273	0.1068
HLLT vs LLLT	0.0105	0.1935	0.0208	0.2791	0.6269	0.8917	0.9332	0.8152
LLHT vs LLLT	0.5771	0.1972	0.1867	0.3708	0.0489	0.1848	0.0303	0.1306

Figure 1. Experiment design. Shade samples of each 10-12 plants are represented by **L** where as non-shaded, high light samples are represented by **H**. This trial was repeated a total of 6 times.
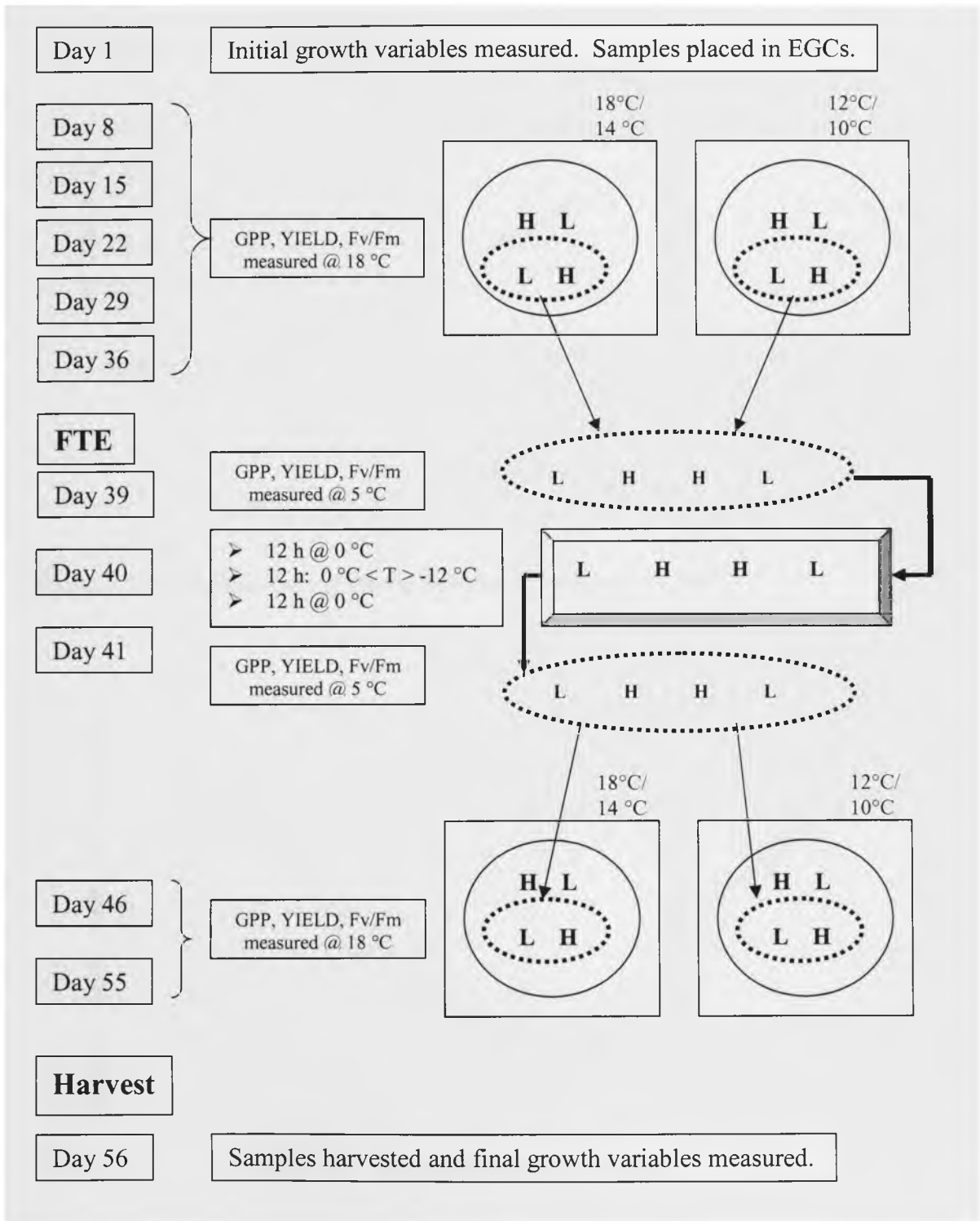
Figure 2. Regression of water content method using dummy samples. n = 32.
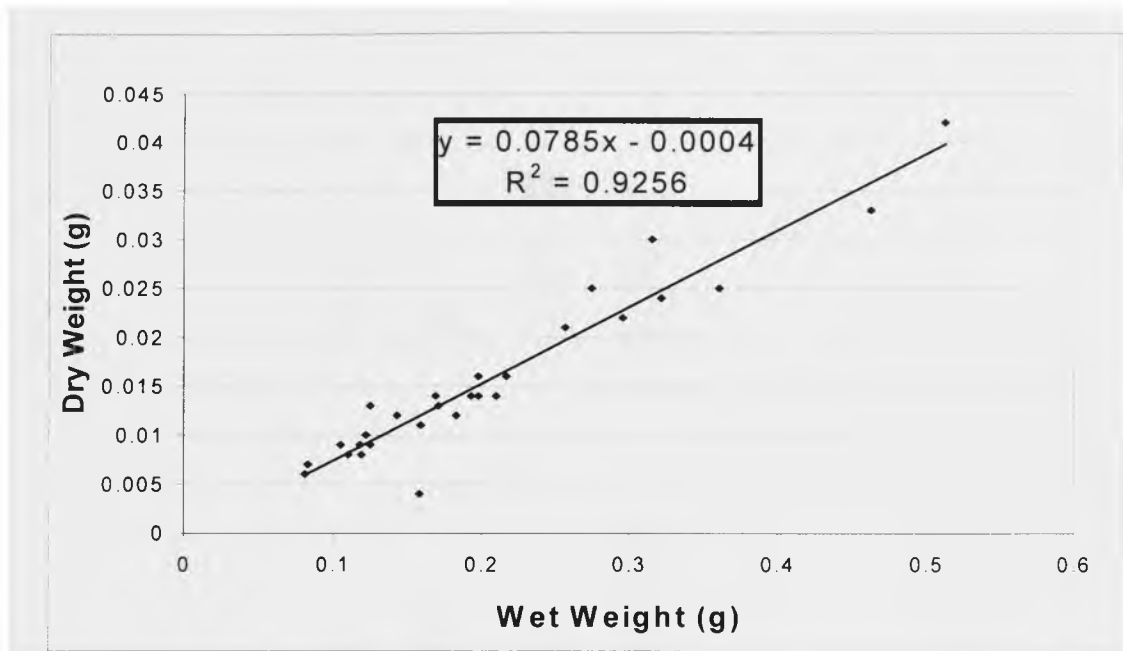

$$y = 0.0785x - 0.0004$$
$$R^2 = 0.9256$$
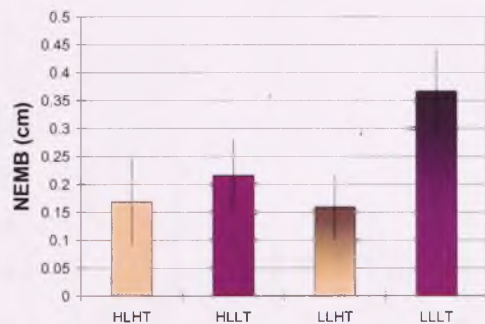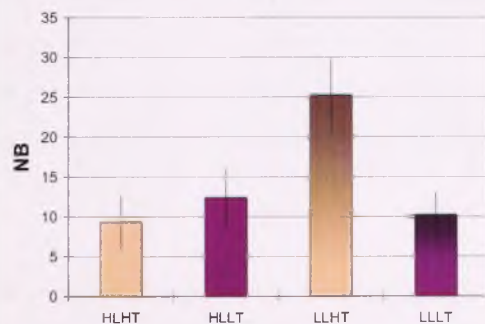
Figure 3. Temperature during the six independent subzero freezes.
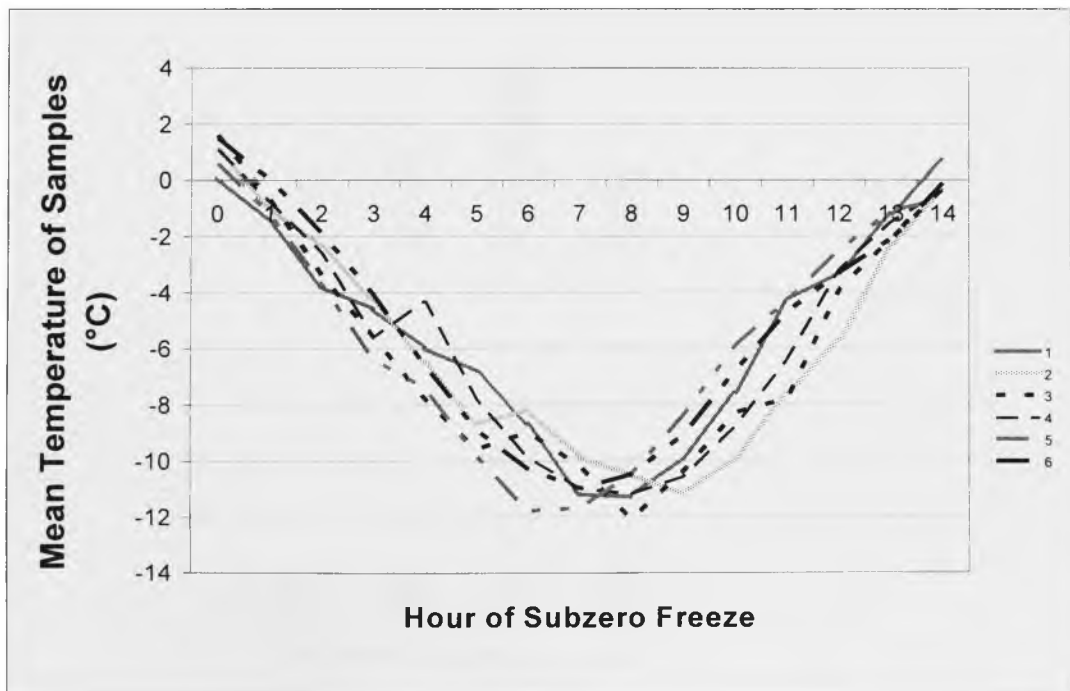
Figure 4. Growth response to four light-temperature environments: a) mean change in net dry weight; b) mean net elongation of main branches c) mean total number of new branches per sample; d) mean total new branch net elongation per sample.
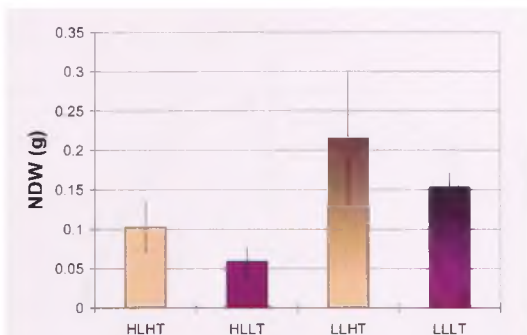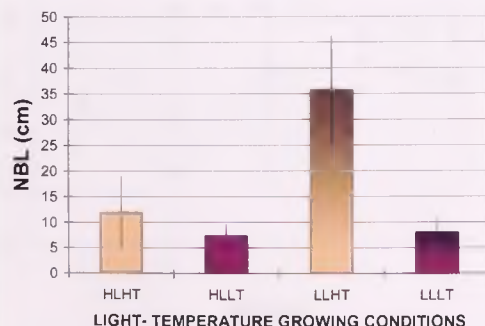
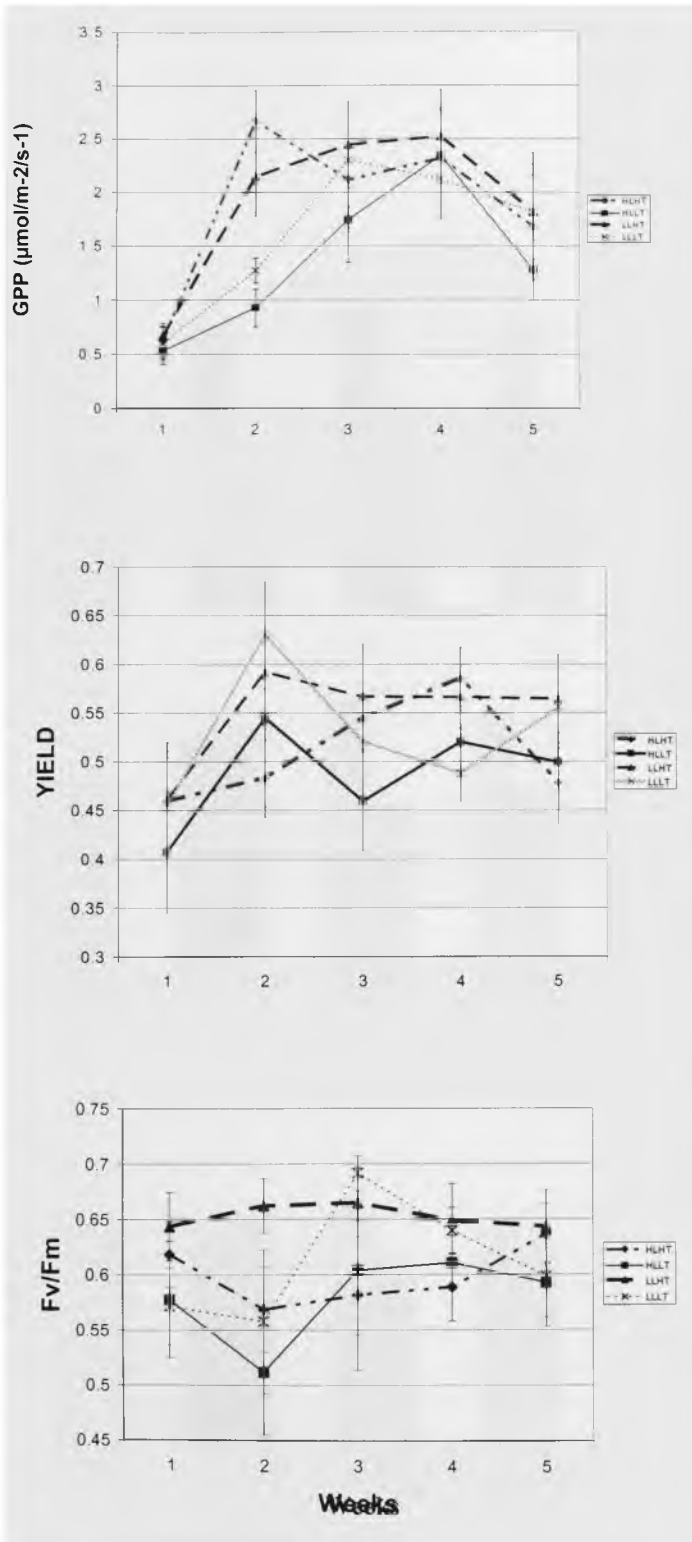Figure 5. Physiological response to four light-temperature environments over 5 weeks: a) GPP (μmol/m^{-2}/s^{-1}); b) Yield; c) F_v/F_m .

a.

b.

c.

46

Figure 6. Short-term freeze effect on four light-temperature acclimation conditions: a) GPP ($\mu mol/m^{-2}/s^{-1}$) b) Yield c) F_v/F_m. All measurements were taken at 6°C.
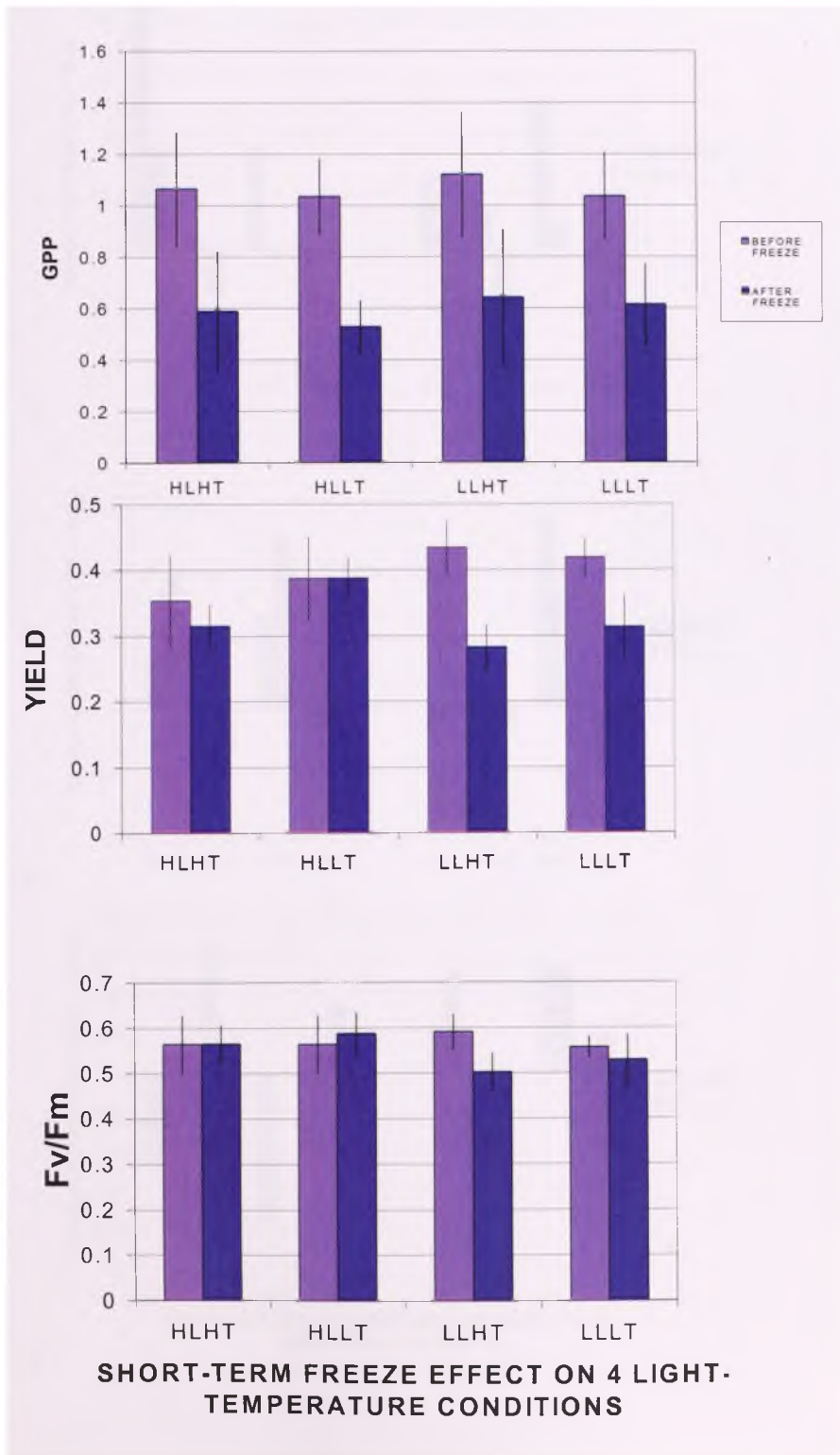
Figure 7. Recovery after FTE shown as measurements 1-Day before FTE minus post Day 5 and post Day 14: a) Gross Primary Productivity ($\mu mol/m^{-2}/s^{-1}$) b) YIELD c) F_v/F_m. All measurements were taken at 18°C.
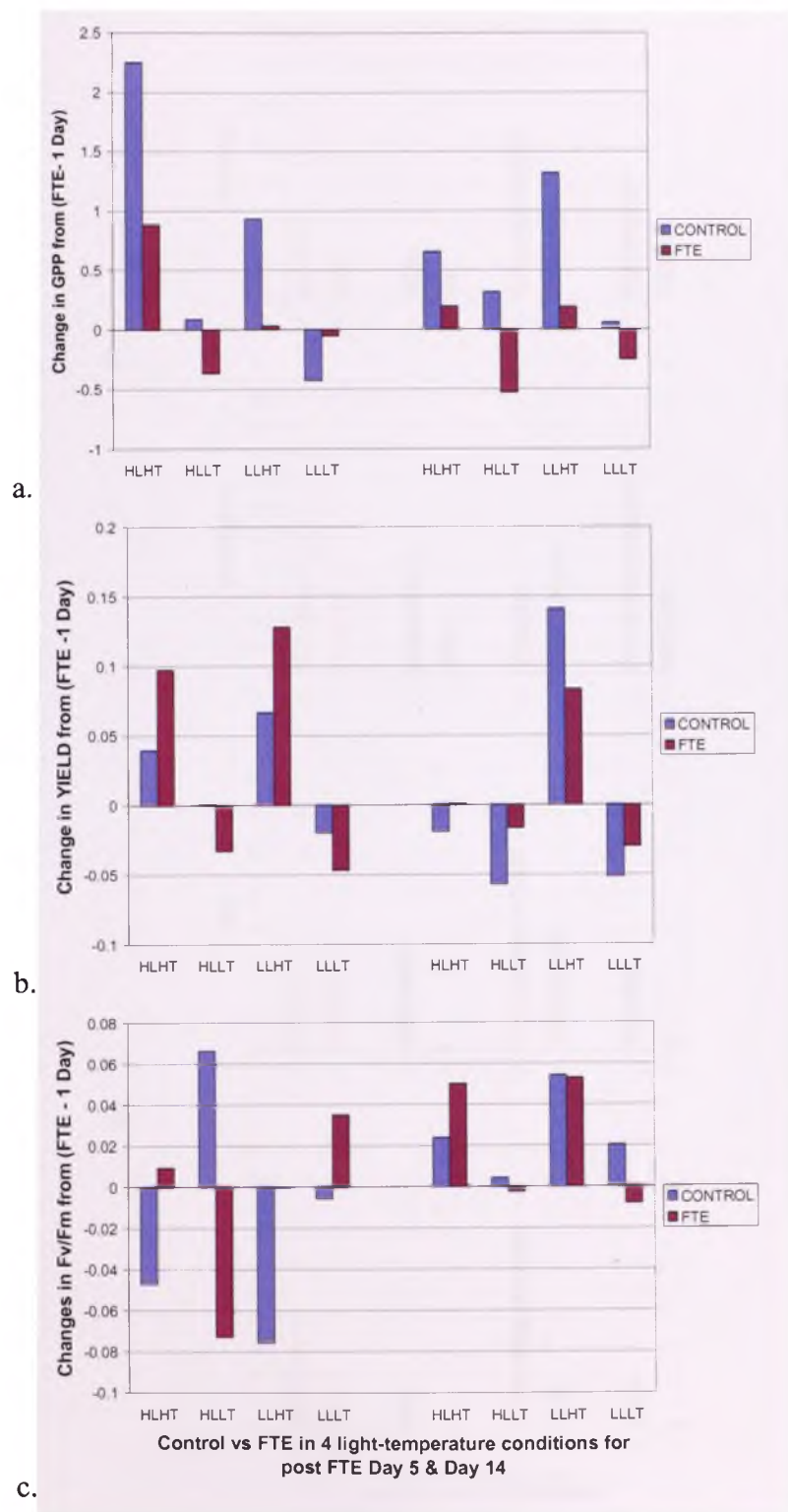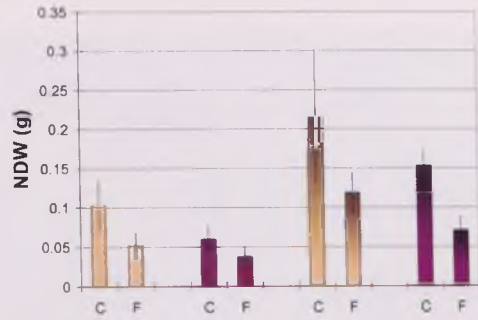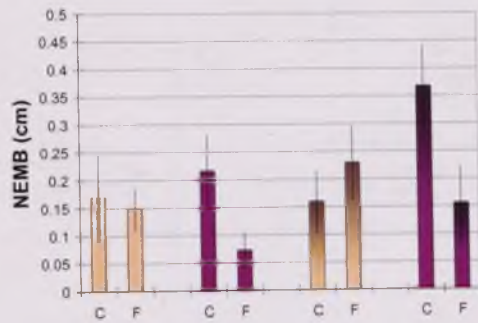

a.

b.

c.

Figure 8. Freeze effect on the growth response under 4 light-temperature environments:
a) mean change in net dry weight; b) mean main branch net elongation; c) mean total
number of new branches per sample; d) mean total new branch net elongation per sample.
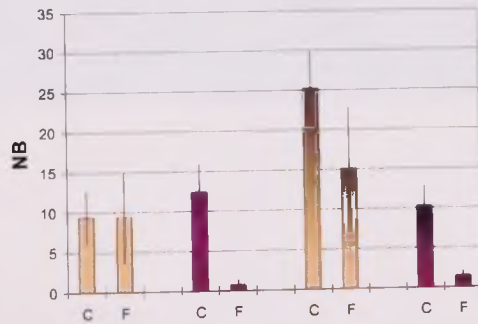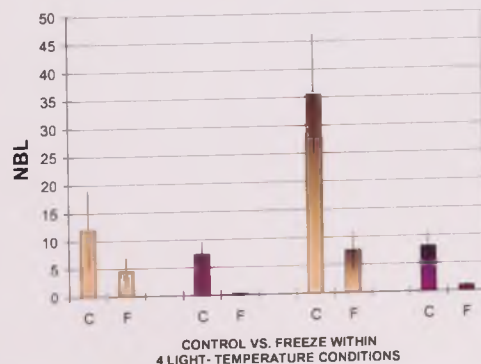
Figure 9. Warmer Climate: *Sphagnum* Loses the Ecosystem Engineering Ability.
Community interactions that have been allowing *Sphagnum* to minimize vegetative cover
may reach a threshold in which the interaction of the physiology of *Sphagnum* and
vascular plants will promote a switch to a LLHT understory as the Arctic warms.
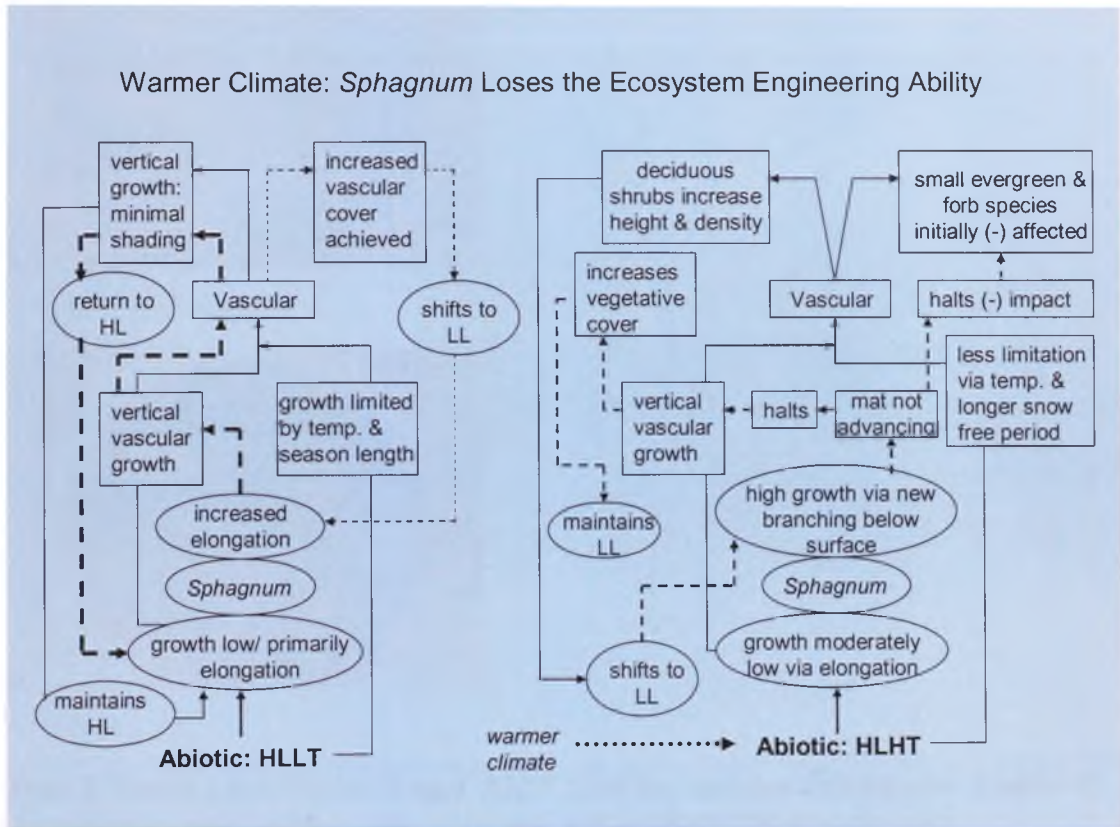
Plate 1: Contrasting color of *Sphagnum* girgensohnii: a) characteristic green color produced in growth chambers; b) brown color with green specks found *in situ*.

a

b

Plate 2: Toolik Lake, Brooks Range, AK: Collection location. *Eriophorum vaginatum, Betula nana, Salix pulchra,* and *Sphagnum* species dominate ground cover.

Plate 3: Freeze-thaw event set-up. Plants samples attached to individual thermocouples are approaching freezing conditions under ice.

Plate 4: Examples of growth from 4 light-temperature environments. *Sphagnum* grown in a) HLHT; b) HLLT; c) LLHT; d) LLLT.

Plate 5: *Sphagnum* branches produced in the low-light, high-temperature environment

Plate 6: Loss of chlorophyll in the tender new shoots due to the freeze-thaw event

Plate 7: *Sphagnum girgensohnii* Russowi under 300X S.E.M. Hyaline cells line the outside of the plant, holding water and nutrients, freezing taking place here first during FTE could serve as protection to the rest of the plant.

300 X

REFERENCES

Aldous, A. R. (2002) "Nitrogen retention by *Sphagnum* mosses: responses to atmospheric nitrogen deposition and drought." Canadian Journal of Botany **80**: 721-731.

Andrus, R. E., Karlin, E.F. & Talbot, S.S. (1992). "Rare and endangered *Sphagnum* species in North America." Biological Conservation **59** (2-3): 247-254.

Arft, A. M., Walker, M.D., Gurevitch, J., Alatalo, J.M., Bret-Harte, M.S., Dale, M., Diemer, M., Gugerli, F., Henry, G.H.R., Jones, M.H., Hollister, R.D., Jonsdottir, I.S., Laine, K., Levesque, E., Marion, G.M., Molau, U., Molgaard, P., Nordenhall, U., Raszhivin, V., Robinson, C.H., Starr, G., Stenstrom, A., Stenstrom, M., Totland, O., Turner, P.L., Walker, L.J., Webber, P.J., Welker, J.M., & Wookey, P.A. (1999). "Responses of tundra plants to experimental warming: meta-analysis of the international tundra experiment." Ecological Monographs **69** (4): 491-511.

Balagurova, N., Drozdov, S., & Grabovik, S. (1996) "Cold and heat resistance of five species of *Sphagnum*." Annales Botanici Fennici **33** (1): 33-37.

Berendse, F., N. V. Breemen, H. Rydin, A. Bultler, M. Heijmans, M. R. Hoosbeek, J.A. Lee, E. Mitchell, T. Saarinen, H. Vansander, B. Wallen. (2001). "Raised atmospheric CO_2 levels and increased N deposition cause shifts in plant species composition and production in *Sphagnum* bogs." Global Change Biology **7**:591-598.

Chapin, F. S. (1992). "Arctic ecosystems in a changing climate: an ecophysiological perspective". *In* Chapin III, F.S., Jefferies, R.L., Reynolds, J.F., Shaver, G.R. & Svoboda, J. (eds.), Arctic Ecosystems in a Changing Climate. San Diego: Academic Press. 35-57.

Clymo, R. S. (1970). "The growth of *Sphagnum*: methods and measurements." Journal of Ecology **58**(1): 13-49.

Clymo, R. S. (1973). "The growth of *Sphagnum*: some effects of environment." Journal of Ecology **61**(3): 849-869.

Clymo, R. S. and Hayward, P. M. (1982). "The ecology of *Sphagnum*." In *Bryophyte Ecology*, (ed. A. J. E. Smith), pp. 229-289. Chapman and Hall, London.

Clymo, R.S. & Duckett, J.G. (1986) "Regeneration of *Sphagnum*." New Phytologist **102**(4): 589-614.

Deltoro, V.I., Calatayud, A., Morales, F., Abadia, A. & Barreno, E. (1999). "Changes in net photosynthesis, chlorophyll fluorescence and xanthophyll cycle interconversions during freeze-thaw cycles in the Mediterranean moss *Leucodon sciuroides*." Oecologia **120**: 499-505.

Dilks, T.J.K. & Proctor, M.C.F. (1975) "Comparative experiments on temperature responses on bryophytes – assimilation, respiration and freezing damage." Journal of Bryology **8**: 317-336.

Dore, M.H.I. (2005). "Climate change and changes in global precipitation patterns: What do we know?" Environmental International **31**(8): 1167-1181.

Gerdol, R., Bonora, A., Marchesini, R., Gualandri, R. & Pancaldi, S. (1998). "Growth response of *Sphagnum capillifolium* to nighttime temperature and nutrient level: mechanisms and implications for global change." Arctic and Alpine Research **30**(4): 388-395.

Gorham, E. (1991). "Northern peatlands: role in the carbon cycle and probable responses to climate warming." Ecological Applications **1**(2): 182-195.

Gorsuch, D.M. & Oberbauer, S.F. (2002). "Effect of mid-season frost and elevated growing season temperature on stomatal conductance and specific zylem conductivity of the arctic shrub, *Salix pulchra*." Tree Physiology **22**: 1027-1034)

Grosvernier, P., Matthey, Y. & Buttler, A. (1997). "Growth potential of three *Sphagnum* species in relation to water table level and peat properties with implications for their restoration in cut-over bogs." Journal of Applied Ecology **34**(2): 471-483.

Gunnarsson, U. & Rydin, H. (2000). "Nitrogen fertilization reduces *Sphagnum* production in bog communities." New Phytologist **147**(3): 527-537.

Harley, P. C., Tenhunen, J.D., Murray, K.J. & Beyers, J (1989). "Irradiance and temperature effects on photosynthesis of tussock tundra *Sphagnum* mosses from the foothills of the Philip Smith Mountains, Alaska." Oecologia. **79**(2): 251-259.

Hastings, S. J., Luchessa, S.A., Oechel, W.C., & Tenhunen, J.D. (1989). "Standing biomass and production in water track drainages of the foothills of the Philip Smith Mountains, Alaska." Holarctic Ecology **12**: 304-311.

Hayward, P.M. & Clymo, R.S. (1983). "The growth of *Sphagnum* – experiments on, and simulation of, some effects of light-flux and water-table depth." Journal of Ecology **71**(3): 845-863.

Heijmans, M., Arp, W.J. & Berendse, F. (2001). "Effects of elevated carbon dioxide and increased nitrogen deposition on bog vegetation in the Netherlands." Journal of Ecology **89**(2): 268-279.

Hicklenton, P.R. & Oechel, W.C. (1976). "Physiological aspects of ecology of *Dicranum-fuscescens* in subarctic. 1. Acclimation and acclimation potential of CO2 exchange in relation to habitat, light, and temperature." Canadian Journal of Botany **54**(10): 1104-1119.

Hinzman, L.D., Bettez, N.D., Bolton, W.R., Chapin, F.S., Dyurgerov, M.B., Fastie, C.L., Griffith, B., Hollister, R.D., Hope, A., Huntington, H.P., Jensen, A.M., Jia, G.J., Jorgenson, T., Kane, D., Klein, D.K., Kofinas, A.D., Lynch, A.H., Llyod, A.H., McGuire, A.D., Nelson, F.E., Oechel, W.C., Osterkamp, T.E., Racine, C.H., Romanovsky, V.E., Stone, R.S., Stow, D.A., Sturm, M., Tweedie, C.E., Vourlitis, G.L., Walker, M.D., Walker, D.A., Webber, P.J., Welker, J.M., Winker, K.S. & Yoshikawa, K. (2005). "Evidence and implications of recent climate change in northern Alaska and other arctic regions." Climate Change **72**: 251-298.

Hobbie, S. E. (1996). "Temperature and plant species control over litter decomposition in Alaskan tundra." Ecological monographs **66**(4): 503-522.

Hobbie, S. E., Shevtsova, A. & Chapin, F.S. (1999). "Plant responses to species removal and experimental warming in Alaskan tussock tundra." Oikos **84**(3): 417-434.

Hobbie, S.E., Schimel, J.P., Trumbore, S.E. & Randerson, J.R. (2000). "Controls over carbon storage and turnover in high-latitude soils." Global Change Biology **6** (Suppl. upp): 196-210.

Hogg, P., Squires, P. & Fitter, A.H. (1995). "Acidification, nitrogen deposition and rapid vegetational change in a small valley mire in Yorkshire." Biological Conservation **71**(2): 143-153.

Hollister, R.D., Webber, P.J. & Tweedie, C.E. (2005). "The response of Alaskan arctic tundra to experimental warming: differences between short- and long-term responses." Global Change Biology **11**(4): 525-536.

Intergovernmental-Panel-of-Climate-Change (2001). "Climate Change 2001: The Scientific Basis" Cambridge, UK: Cambridge University Press.

Intergovernmental-Panel-of-Climate-Change (2007). "The Physical Science Basis" Cambridge, UK: Cambridge University Press.

Jauhiainen, J., Silvola, J. & Vasander, H. (1998). "The effects of increased nitrogen deposition and CO_2 on *Sphagnum angustifolium* and *S. warnstorfii*." Annales Botanici Fennici **35**(4): 247-256.

Jorgenson, M.T., Shur, Y.L. & Pullman, E.R. (2006) "Abrupt increase in permafrost degradation in Arctic Alaska." Geophysical Research Letters 33(2): L02503.

Kane, D. L., Hinzman, L.D., Woo, M. & Everett, K.R. (1992). "Arctic hydrology and climate change." Arctic Ecosystems in a Changing Climate. F. S. Chapin, III, *et al.* San Diego, Academic Press, Inc: 35-57.

Keeling, C. D., Chin, J.F.S. & Whorf, T.P. (1996). "Increased activity of northern vegetation inferred from atmospheric CO_2 measurements." Nature 382(6587): 146-149.

Kennedy, A.D. (1993). "Photosynthetic response of the Antarctic moss *Polytrichum-alpestre* Hoppe to low-temperatures and freeze-thaw stress." Polar Biology 13(4): 271-279.

Koprivnjak, J.F. & Moore, T.R. (1992). "Sources, sinks, and fluxes of dissolved organic carbon in subarctic fen catchments." Arctic and Alpine Research 24 (3): 204-210.

Kullman, L. (2002) "Rapid recent range-margin rise of tree and shrub species in the Swedish Scandes." Journal of Ecology 90(1): 68-77.

Longton, R.E. (1992). "The role of bryophytes and lichens in terrestrial ecosystems." In *Bryophtes and Lichens in a Changing Environment*. (ed. J. W. Bates and A. M. Farmer) 32-76 Oxford University Press, Oxford.

Lovelock, C.E., Jackson, A.E., Melick, D.R. & Seppelt, R.D. (1995) "Reversible photoinhibition in antarctic moss during freezing and thawing." Plant Physiology 109: 955-961.

Mäkipää, R. & Heikkinen, J. (2003) "Large-scale changes in adundance of terricolous bryophytes and macrolichens in Finland." Journal of Vegetation Science 14: 497-508.

Malmer, N., Svensson, B.M. & Wallen, B. (1994). "Interactions between *Sphagnum* mosses and field layer vascular plnts in the development of peat-forming systems." Folia Geobotanica & Phytotaxonomica 29(4): 483-496.

Marion, G.M., Bockheim, J.G. & Brown, J (1997). "Arctic soils and the ITEX experiment." Global Change Biology 3(Suppl. 1): 33-43.

Maxwell, B. (1992). Arctic Climate: Potential for Change under Global Warming. Arctic Ecosystems in a Changing Climate. F. S. Chapin, III, *et al.* San Diego, Academic Press, Inc: 11-34.

Mazur, P. (1969). "Freeze injury in plants." <u>Annual Review of Plant Physiology</u> **20**: 419-448.

Mitchell, E.A., Buttler, A., Grosvernier, P., Rydin, H., Siegenthaler, A. & Gobat, J.M. (2002) "Contrasted effects of increased N and CO_2 supply on two keystone species in peatland restoration and implications for global change." <u>Journal of Ecology</u> **90**: 529-533.

Moorhead, D.L. & Reynolds J.F. (1993). "Effects of climate change on decomposition in Arctic tussock tundra: A modeling synthesis." <u>Arctic and Alpine Research</u> **25**(4): 403-412.

Muller, C.H. (1952). "Plant succession in arctic heath and tundra in northern Scandinavia." <u>Bulletin of the Torrey Botanical Club</u> **79**: 296-309.

Mulligan, R.C. & Gignac, L.D. (2001) "Bryophyte community structure in a boreal poor fen: reciprocal transplants." <u>Canadian Journal of Botany</u> **79**: 404-411.

Mulligan, R.C. & Gignac, L.D. (2002) "Bryophyte community structure in a boreal poor fen II: interspecific competition among five mosses." <u>Canadian Journal of Botany</u> **80**(4): 330-339.

Murray, K. J., Tenhunen, J.D. & Kummerow, J. (1989) "Limitation on *Sphagnum* growth and net primary production in the foothills of the Philip Smith Mountains, Alaska." <u>Oecologia</u> **80**: 256-262.

Murray, K. J., Tenhunen, J.D. & Nowak, R.S. (1993). "Photoinhibition as a control on photosynthesis and production of *Sphagnum* mosses." <u>Oecologia</u> **96**(2): 200-207.

Oberbauer, S.F. & Starr, G. 2002. The role of anthocyanins for photosynthesis of Alaskan arctic evergreens during snow melt. <u>Advances in Botanical Research</u> **37**: 129-145.

Oberbauer, S.F., Starr, G. & Pop, E.W. 1998. "Effects of extended growing season and soil warming on carbon dioxide and methane exchange of tussock tundra in Alaska." <u>Journal of Geophysical Research</u> **103**:29075-29082

Oechel, W. C., Hastings, S.J., Vourlitis, G., Jenkins, M., Riechers, G. & Grulke, N. (1993). "Recent change of Arctic tundra ecosystems from a net carbon dioxide sink to a source." <u>Nature</u> **361**(6412): 520-523.

Oechel, W.C. & Vourlitis, G.L. (1994). "The effects of climate change on land-atmosphere feedbacks in arctic tundra regions." <u>Trends in Ecology & Evolution</u> **9**(9): 324–329

Ohlson, M., Okland, R.H., Nordbakken, J. & Dahlberg, B. (2001). "Fatal interactions between Scots pine and *Sphagnum* mosses in bog ecosystems." <u>Oikos</u> **94**: 425-432.

Pannewitz, S., Schlensog, M., Green, T.G.A., Sancho, L.G. & Schroeter, B. (2004). "Are lichens active under snow in continental Antarctica?" <u>Oecologia</u> **135**: 30-38.

Repo, T., Hänninen, H., & Kellomäki, S. (1996). "The effects of long-term elevation of air temperature and CO_2 on the frost hardiness of Scots pine." <u>Plant, Cell & Environment</u> **19**: 209-216.

Roy, V., Ruel, J.C. & Plamondon, A.P. 2000. "Establishment, growth and survival of natural regeneration after clearcutting and drainage on the forested wetlands." <u>Forest Ecology and Management</u> **129**: 253-267.

Searles, P.S., Flint, S.D., Diaz, S.B., Rousseaux, M.C., Ballare, C.L. & Caldwell, M.M. (2002) "Plant response to solar ultraviolet-B radiation in a southern South American *Sphagnum* peatland." <u>Journal of Ecology</u> **90**: 704-713.

Serreze, M.C., Walsh, J.E., Chapin, F.S., Osterkamp, T., Dyurgerov, M., Romanovsky, V., Oechel, W.C., Morison, J., Zhang, T. & Barry R.G. (2000). "Observational evidence of recent change in the northern high-latitude environment." <u>Climate Change</u> **46**(1-2): 159-207.

Shaver, G. and J. Laundre. Daily Soil Temperature and Meteorological Data for Sites at Toolik Lake Alaska. 2003. Boulder, CO: National Snow and Ice Data Center/World Data Center for Glaciology. Digital media.

Skre, O. & Oechel, W.C. (1981). "Moss functioning in different taiga ecosystems in interior Alaska." <u>Oecologia</u> **48**: 50-59.

Starr, G., Oberbauer, S.F. & Pop, E.W. (2000). "Effects of lengthened growing season and soil warming on the phenology and physiology of *Polygonium bistorta*." <u>Global Change Biology</u> **6**(3): 357-369.

Stein, J., Proulx, S. & Levesque, D. (1994). "Forest floor frost dynamics during spring snowmelt in a boreal forested basin." <u>Water Resources Research</u> **30**(4): 995-1007.

Svensson, B. (1995). "Competition between *Sphagnum fuscum* and *Drosera rotundifolia*: A case of ecosystem engineering." <u>Oikos</u> **74**(2) 205-212.

Taulavuori, K.M.J., Laine, K., Taulavuori, E.B., Pakonen, T. & Saari, E. (1997) "Accelerated dehardening in the bilberry (*Vaccinium myrtillus* L.) induced by a small elevation in air temperature." <u>Environmental Pollution</u> **98**: 91-95.

Taulavuori, K.M.J., Taulavuori, E.B., Skre, O., Igeland, B. & Laine, K.M. (2004) "Dehardening of mountain birch (*Betula pubescens* ssp. *czerepanovii*) ecotypes at elevated winter temperatures." New Phytologist **162**: 427-436.

Tenhunen, J.D., Lange, O.L., Hahn, S.C.], Siegwolf, R. & Oberbauer S.F. (1992) "The ecosystem role of poikilohydric tundra plants." *In* Chapin III, F.S., Jefferies, R.L., Reynolds, J.F., Shaver, G.R. & Svoboda, J. (eds.), Arctic Ecosystems in a Changing Climate. San Diego: Academic Press. 193-237.

Van Breemen, N. (1995). "How *Sphagnum* bogs down other plants." Trends in Ecology & Evolution **10**(7): 270-275.

Van Der Heijden, E., Verbeek, S.K. & Kuiper, P.J.C. (2000). "Elevated atmospheric CO_2 and increased nitrogen deposition: effects on C and N metabolism and growth of the peat moss *Sphagnum recurvum* P. Beauv. var. *mucronatum* (Russ.) Warnst." Global Change Biology **6**(2): 201-212.

Vavrus, S., Walsh, J.E., Chapman, W.L., Portis, D. (2006). "The behavior of extreme cold air outbreaks under greenhouse warming." International Journal of Climatology **26**: 1133-1147.

Vitt, D.H., Halsey, L.A. & Zoltai, S.C. (1994). "The bog landforms of continental western Canada in relation to climate and permafrost patterns." Arctic and Alpine Research **26**(1): 1-13.

Waddington, J. M., Rotenberg, P.A. & Warren, F.J. (2001). "Peat CO_2 production in a natural and cutover peatland: Implications for restoration." Biogeochemistry **54**(2): 115-130.

Walker, D. A., Binnian, E., Evans, B.E., Lederer, N.D., Nordstrand, E., Webber, P.J. (1989). "Terrian, vegetation, and landscape evolution of the R4D research site, Brooks Range Foothills, Alaska." Holarctic Ecology **12**: 238-261.

Walker, M.D., Wahren, C.H., Hollister, R.D., Henry, G.H.R., Ahlquist, L.E., Alatalo, J.M., Bret-Harte, M.S., Calef, M.P., Callaghan, T.V., Carroll, A.B., Epstein, H.E., Jonsdottir, I.S., Klein, J.A., Magnusson, B., Molau, U., Oberbauer, S.F., Rewa, S.P., Robinson, C.H., Shaver, G.R., Suding, K.N., Thompson, C.C., Tolvanen, A., Totland, P.L., Turner, P.L., Tweedie, C.E., Webber, P.J. & Wookey, P.A. (2006) "Plant community responses to experimental warming across the tundra biome." Proceedings pf the National Academy of Sciences of the United States of America **103**(5): 1342-1346.

Walker, M.D., Walker, D.A. & Auerbach, N.A. (1994). "Plant- communities of a tussock tundra landscape in the Brooks Range Foothills, Alaska." Journal of Vegetation Science **5**(6): 843-866.

Woo, M.K. & Young, K.L. (2006). "High Arctic wetlands: their occurrence, hydrological characteristics and sustainability." Journal of Hydrology **320**(3-4): 432-450.