The responses of temperate and sub-arctic bryophytes to changing environmental conditions

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MSc Thesis

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October 2014

Abstract

Climate change presents a serious threat to many global ecosystems. Warming is predicted to be greatest at high latitudes, where increased temperatures are expected to lead to a longer growing season and an increased incidence of potentially damaging winter freeze-thaw events. The impact of these environmental changes on the native flora is not yet fully understood. The bryophytes are an ancient group of non-vascular plants which often form a large proportion of the plant community in the arctic and boreal regions. The response of these organisms to climate change has often been overlooked, despite the significant role they play in carbon uptake and storage in many ecosystems.

The study used infra-red gas analysis to measure the change in net photosynthesis and respiration rates in several bryophyte species in response to changing microclimate. Experiments were conducted under controlled laboratory conditions using samples of the common moss species *Polytrichum juniperinum*, *Hylocomium splendens* and *Aulacomnium palustre*, collected from the North Pennines, UK. Additional measurements of gas exchange rates in the species *Hylocomium splendens*, *Aulacomnium turgidum* and *Tomentypnum nitens* were taken at a site near Inuvik, NWT in the Canadian arctic.

The results of the study support the existing evidence that many bryophyte species are adapted to photosynthesis at low temperatures (<10°C), and are therefore unlikely to benefit from an increase in summer temperatures in terms of carbon uptake and growth. The study also found evidence that cycles of freezing and thawing during the winter and early spring cause a significant reduction in carbon uptake in both *P. juniperinum* and *H. splendens* compared to a single period of sub-zero temperatures. This damage was reduced in *P. juniperinum* when the plants were air-dry at the time of freezing. The data arising from this study are important to improve existing models of carbon exchange in bryophyte-dominated ecosystems.

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Acknowledgments

My thanks go firstly to my supervisor Dr Bob Baxter for all his support and encouragement over the past year. From his initial enthusiasm for the project, which never waned, to his tireless help and guidance throughout, he has been a fantastic mentor and a privilege to work with.

I am enormously grateful to Dr Lorna Street for her valuable input at every stage of the project, and her assistance both in the field, and with the subsequent data analysis. My time in the Canadian arctic is an experience I will never forget, and for that I must also thank Prof Phil Wookey and Dr Mark Cooper for their support, company, and endless supplies of chocolate.

This research would not have been possible without the support of project HYDRA, the AURORA institute in Inuvik, and funding from the Grevillea trust, thank you.

Finally, I would like to thank all my friends and colleagues at the University of Durham for a great year, and my family for their love and patience. I couldn't have done it without you.

Chapter one: Introduction and literature review

Introduction

Since the early 20th Century the mean surface temperature of the Earth has increased by 0.8°C. This rise has been primarily due to the effects of anthropogenic climate forcing, through the release of greenhouse gasses, including carbon dioxide (CO_2) and methane (CH_4) (IPCC, 2007). The IPCC fourth assessment report (IPCC, 2007), predicts that over the coming century the global climate is likely to warm by a further 2-4°C, as the rate at which these gasses are released into the atmosphere continues to increase. Climate models predict that future climate change will affect different areas of the globe in different ways. For example, continental areas are predicted to warm to a greater extent than the oceans, and the Arctic to warm more than equatorial regions. In addition to global temperatures, patterns of precipitation are also predicted to change. Many areas, including the Arctic, are predicted to receive increased precipitation, while others, including Western Europe, are predicted to experience reduced precipitation (IPCC, 2007).

Climate change is already having a measurable impact on many global ecosystems. One major effect of climate change, particularly at higher latitudes, has been the earlier onset of spring events. During the last 30 years the growing season in the northern hemisphere has increased by 3-5 days per decade (Genet et al., 2013). An earlier onset of spring bud break provides the opportunity for increased plant growth over the summer season. However an early period of warm weather followed by late frosts can cause severe damage to new shoots which would otherwise have remained dormant until later in the year. For example, in the Arctic, incidents of extreme warming for a few days in the middle of winter can melt the insulating snow layer, leaving the plants vulnerable to damage when temperatures subsequently fall back well below freezing point (Bokhorst et al., 2011). Understanding how these different factors associated with climatic warming affect the overall survival and growth of plants is necessary understand the ways in which climate change may affect these communities as a whole.

The contribution of bryophytes to the global carbon balance

While much research has been undertaken to investigate the responses of animals and higher plants to climate change, the responses of bryophytes (mosses & liverworts) and lichens have generally been largely overlooked (Street et al., 2012). The bryophytes are an ancient group of non-vascular plants which may form a significant proportion of the plant community, particularly in high-latitude and high-altitude environments where vascular plants struggle to survive. It is therefore important that these organisms are not ignored if these ecosystems are to be fully described, and the potential impacts of climate change upon such ecosystems understood (Douma et al., 2007; Turetsky et al., 2012).

Although bryophytes have been clearly observed as a dominant form of vegetation in arctic and upland ecosystems, until recently little was known about the relative contribution they made to the overall carbon budget of these ecosystems. Recent work has begun to separate the contribution of mosses from that of vascular plants. In northern Sweden mosses are estimated to be responsible for an average of 60% of annual ecosystem carbon uptake across the landscape (Douma et al., 2007). The relative contribution of bryophytes compared to vascular plants is often greater during the spring and autumn, when vascular plant leaves have either not fully developed, or are entering senescence. For example, between March and May carbon dioxide uptake by *Polytrichum pilferum* in northern Sweden is up to three times that of vascular plants (Street et al., 2012). The loss of moss cover in the tundra environment would therefore be expected to have severe consequences for the carbon balance of these ecosystems (Street et al., 2013).

The majority of research on the responses of mosses to climate change has been conducted in the arctic and alpine tundra regions, as these environments are some of those most heavily dominated by bryophytes (Bates et al., 2005; Lang et al., 2012). Bryophytes are however also a dominant form of vegetation in many other ecosystems, including boreal woodland, and the moorland and peatland ecosystems of temperate latitudes. Below the treeline bryophytes make a smaller, but still significant, contribution to the ecosystem carbon balance. In the black spruce forests of central Canada, for example, moss photosynthesis is estimated to contribute

approximately 13% of the total ecosystem gross production (Swanson and Flanagan, 2001). In total, moss-dominated northern hemisphere boreal and temperate peatlands are estimated to sequester an average of 23g C m^{-2} y^{-1} from the atmosphere. Values as high as 102g C m^{-2} y^{-1} have been reported from UK peat bogs, where conditions are warmer and wetter than comparable continental ecosystems (Billett et al., 2010).

Climate change has the potential to significantly alter these ecosystems by changing the competitive balance between bryophytes and vascular plants, or creating conditions which are detrimental to both. Such a shift could, in turn, have major implications for the global carbon balance (Turetsky et al., 2012). If warming causes increased plant growth and carbon storage, this may help to negate some of the effects of anthropogenic carbon release. If however plant growth is reduced, a negative feedback cycle could contribute to further warming (IPCC, 2007). In many northern ecosystems, warming is expected to significantly increase the rate of soil carbon release. Therefore, even if the vegetation of these ecosystems is relatively unaffected by climate change, the net result may be a fall in net carbon uptake (Billett et al., 2010). In order to predict the likely consequences of climate change in these ecosystems, it is necessary to understand the responses of both vascular plants and bryophytes to a range of potential future climate scenarios.

The effect of changing temperature on Bryophytes

In arctic regions where summer temperatures typically remain low, several studies have shown mosses responding positively to summer warming. Warming of just 1°C above ambient during the summer months caused a significant increase in shoot length and overall biomass production in *Sphagnum fuscum* (Dorrepaal et al., 2004). In northern Canada, experimental warming of 1°C was associated with a 6% increase in overall bryophyte cover after 15 years; however this was coupled with a 3.5% loss of lichen cover (Hudson and Henry, 2010). In Alaska, increasing temperature has been correlated with increased abundance of the common mosses *Sphagnum girgensohnii*, *Hylocomium splendens* and *Pleurozium schreberi*. However the abundance of many other bryophytes, including rarer mosses and lichens, is negatively correlated with increasing temperature (Lang et al., 2012).

In addition to the direct effects of summer warming on bryophyte-dominated ecosystems, mosses may also benefit from the indirect effects of warming. It has been demonstrated that the moss carpet plays an important role in assimilating the carbon dioxide released from wet soils by respiration of micro-organisms (Sommerkorn et al., 1999). Increased temperatures are predicted to increase soil CO_2 flux, creating an environment where elevated ambient CO_2 at the moss surface could cause elevated levels of net photosynthesis. Net photosynthesis of *H. splendens* in a sub-arctic forest in northern Sweden was found to be 2-3 x higher at an ambient CO_2 concentration of 600ppm compared to 350ppm (Sonesson et al., 1992). These values are comparable to measured CO_2 concentrations at the moss surface *in situ,* which rose to 730ppm in the height of summer.

Whilst average winter air temperatures in the sub-arctic tundra can be as low as minus 40°C, in areas where the ground is covered in a deep layer of snow, the temperature at ground level remains close to 0°C (Olsson et al., 2003). It has recently been demonstrated that where a film of unfrozen water is present, and light is able to penetrate the snowpack, some mosses are capable of significant rates of photosynthesis beneath snow cover (Zotz and Rottenberger, 2001). In contrast, vascular plants typically require higher temperatures and light intensities for photosynthesis to occur (Larsen et al., 2007). The mosses which live under these conditions are rarely exposed to the extreme cold of the arctic winter, and the subnivian environment allows them to remain metabolically active throughout the winter. It is this protection which allows species typically found in temperate regions to survive the arctic winter. In experiments where the snow is artificially removed under these conditions mosses show significantly reduced survival and growth (Bjerke et al., 2011; Bokhorst et al., 2011).

In recent years the number of midwinter warming events in the Arctic has increased, an effect of climate change which is expected to continue in the future. During these events the air temperature can rise from far below freezing to above 0°C in a few hours (Olsson et al., 2003; Bokhorst et al., 2011). This causes rapid snow-melt, exposing plants to higher temperatures and light intensities. Under these conditions mosses can respond with a rapid increase in photosynthetic rate within minutes.

Within days the plants begin to develop new freeze-susceptible shoots, responding as though it were spring. (Bjerke et al., 2013). When the warming event passes, the mosses are left exposed to freezing air temperatures. In the Arctic the majority of snowfall generally occurs in the autumn, with little during the winter or early spring (Olsson et al., 2003). The mosses are therefore likely to remain exposed for the remainder of the winter. This exposure has been shown to severely damage their photosynthetic capability. In the field, patches of *Hylocomium splendens* showed significant reductions in growth and photosynthetic rate of up to 50% in the summer following an artificially induced winter warming event (Bjerke et al., 2011). Recovery from laboratory-induced freeze-thaw events is slower when the mosses are frozen at lower temperatures, and when they are subjected to multiple cycles of freezing and thawing compared to a single warming event (Kennedy, 1993).

In temperate regions where snow cover is erratic through the winter the plants do not have the benefit of the protection of a deep, long-lived snowpack. Whilst air temperatures do not drop as low as in the arctic, night-time minima of -10° C or lower are not uncommon in many regions including the UK (Met Office, 2012). Few studies have been undertaken on the effect that these sudden dips in temperature may have on the native bryophyte species. As in the Arctic, bryophytes in these regions respond to unseasonably warm temperatures with rapid growth and development, leaving them potentially vulnerable to late frosts. Research into the effects of rapid freeze-thaw cycles on these species is therefore equally applicable to arctic and temperate environments.

In warmer regions where winter temperatures rarely fall below freezing and the risk of late spring frosts is minimal, bryophytes may benefit from winter warming. The ability to photosynthesise at relatively low temperatures and light intensities places them in the ideal position to take advantage of cool and wet winter conditions in regions where summers are likely to be hot and dry; conditions that are detrimental to moss growth (Zotz and Rottenberger, 2001; Bates et al., 2005). The response of individual bryophyte species to warming is therefore dependent upon a range of environmental and temporal factors, including the competitive dynamics of the ecosystem, and can vary significantly between sites and species (Bates et al., 2005; Davey and Rothery, 1997).

The effect of changing water availability on bryophytes

Mosses and other bryophytes differ from vascular plants in that bryophytes lack xylem vessels and true roots; instead they obtain water and nutrients by absorbing them directly through the leaves over the whole surface of the plant (Dilks and Proctor, 1979). This strategy allows them to grow directly on surfaces which cannot support higher plants, for example bare rock, tree stumps, and low-quality soils. A lack of vascular system for the transport and storage of water means that mosses can only grow in damp areas where water is abundantly available for at least part of the year. The majority of moss species also lack a cuticle on their leaves. This, in combination with their small size, means that water loss in dry conditions is often rapid (Dilks and Proctor, 1979).

When the moisture content of mosses is reduced below an optimum, the net photosynthetic rate also falls (Williams and Flanagan, 1996). During periods when water is not available, many species are able to survive desiccation by entering a form of stasis where metabolism is halted until water once again becomes available (Davey, 1997). In a laboratory study, air-dried samples of *Racomitrium lanuginosum*, *Anomodon viticulosus* and *Rhytidiadelphus loreus*, all demonstrated a full recovery of maximum net photosynthesis within 24h of re-wetting (Proctor and Smirnoff, 2000). Therefore, while mosses are very sensitive to water loss in the short term, they do not suffer any long term harm from desiccation, and remain readily able to take advantage of an increased water supply.

Many studies investigating the effects of climate change on mosses have found that the response to warming varied considerably depending on the availability of water (Uchida et al., 2002; Williams and Flanagan, 1996). In the common Arctic moss *Sanionia uncinata,* net photosynthesis was found to be constant over a wide temperature range (7-23° C) when the moss was fully hydrated (Uchida et al., 2002). The study found that the large inter-annual variation in the net primary productivity of this species was almost entirely due to water availability. In Alaska, summer net photosynthesis of mosses was found to be higher during years when water content was favourable for growth in the early season, compared to years with greater precipitation overall, but drier conditions during the spring (Skre and Oechel,

1981). These findings suggest that even in colder regions, moss growth is limited by water availability rather than temperature.

Studies have shown that mosses in temperate regions are most productive during the spring and autumn, taking advantage of the high precipitation levels and cool temperatures. Growth rates are then reduced during the warmer, but drier, summer period (Bates et al., 2005). A study of a moss community growing on a stone wall in Germany found that net productivity of all species was highest in the autumn and lowest in the summer, with winter and spring rates intermediate and more variable between species. Rates of photosynthesis in all seasons were highly variable between years depending on weather conditions (Zotz and Rottenberger, 2001). In areas where annual precipitation is predicted to decrease as a consequence of climate change, including much of Western Europe, warming is likely to reduce the growth and production of mosses during the formerly productive cooler months.

The response of mosses to freezing stress is also highly dependent on moisture availability. For example, mosses in a desiccated state show significantly less damage and recover more rapidly from freezing at very low temperatures (Kennedy, 1993). The similarity between drought and freezing stress has been well documented in vascular plants. The physiological responses induced by both stresses share many of the same biochemical pathways and produce similar physiological effects. For example, the accumulation of compatible solutes inside cells lowers the water potential of the inter-cellular fluid, both reducing water loss from the cell and lowering its freezing point (Kasuga et al., 1999; Burke et al., 1976).

While snow cover can help to insulate mosses from frigid arctic winters, increased precipitation during the spring, when temperatures regularly fluctuate above and below freezing, may exacerbate the effects of freezing stress on mosses (Kennedy, 1993). In xeric habitats, the natural desiccation response helps to protect mosses from freezing damage (Schlensog et al., 2004). In addition to this, mosses which are metabolically inactive due to drought, will not respond to warming with new growth which may be vulnerable to later freezing (Bjerke et al., 2013). While this may be a disadvantage when spring arrives 'for real', during periods of freeze-thaw conditions it provides a vital defence. These studies demonstrate that there are

multiple factors which influence how bryophytes may respond to climate change. Further study is necessary to determine the overall impact that these combined factors may have on different species.

Context and justification for the study

Whilst research into the effects of climate change on bryophytes is still in the early stages, it is clear that environmental change is having a significant impact on the physiology and ecology of these important plants. Perhaps unsurprisingly it has been found that different bryophyte species show radically different responses to climate change (Lang et al., 2012). These organisms should therefore not be treated as a single group as they often are in ecological studies, but instead studied separately and in competition with one another (Turetsky et al., 2012). It is important that quantitative data are collected on the responses of a wide range of species to different possible future climate scenarios if we are to understand the potential impacts of climate change on these communities.

The discovery that many bryophytes are photosynthetically active for a longer period of the year than vascular plants opens up an important area for research. Little is known about the photosynthetic capabilities of bryophytes at low temperatures (-5 to +5 °C), and how they may respond to an earlier spring thaw (Street et al., 2012). A number of challenges exist in this area of research. For example, due to the severity of winter conditions in high latitude and altitude environments, field measurements during the winter and early spring are almost impossible to make. It is therefore desirable to conduct much of this research in the laboratory, under controlled environmental conditions. Accurately quantifying the gas exchange capability of bryophytes at these temperatures is a key aim of this study.

The majority of research on the responses of bryophytes to climate change has been conducted in the Arctic; however bryophytes also make a significant contribution to the carbon cycle in temperate and tropical environments (Bates et al., 2005; Billett et al., 2010). Although there are some bryophyte species which grow only in Arctic environments, many of the most common are found over a very large climatic range,

from the arctic and alpine tundra to temperate and even sub-tropical regions. The challenges faced by these plants as a result of climate change differ from the challenges faced by arctic populations. This study will investigate populations of common bryophyte species from both a sub-arctic tundra site in northern Canada, and a temperate upland environment in the UK.

Aims and objectives

The aim of this study was to investigate the response of a range of dominant boreal bryophyte species to altered microclimate, with a focus was on measuring changes in carbon exchange between plant and atmosphere in response to changes in ambient temperature and water availability. A significant challenge of the project was to measure rates of photosynthesis and respiration at temperatures close to the freeze-thaw boundary (< +5 °C) using infra-red gas analysis. The data arising from the study are important to improve existing models of carbon exchange in bryophyte-dominated ecosystems, and will be applicable to both sub-arctic and temperate environments. The aims of the study were addressed by postulation of the following hypotheses:

1: At temperatures in excess of freezing, climate warming will result in enhanced photosynthetic activity of the moss component, so long as water supply is sufficient.

2: Cycles of freezing and thawing during the winter period will negatively impact the photosynthetic capabilities of mosses.

The following chapters include a description of the study methodology (chapter two), the results of the study (chapter three), and a discussion of these results (chapter four).

Chapter two: Methodology

Field sites

Two different field sites were used in this study, one in the United Kingdom and one in Canada. The UK site was located in the North Pennine hills near Stanhope, County Durham (54° 48' 10" N 2° 2' 34" W) (Figure 1). The landscape at this site is open heather moorland, and the ecosystem has a significant bryophyte component, with mosses forming a thick carpet beneath the heather shrub layer. The site has a temperate oceanic climate, with an average daily maximum temperature in July of 20 °C, and an average minimum in January of 0 °C (Met Office, 2012). All laboratory experiments using moss material collected from the UK site were undertaken at the University of Durham, UK.

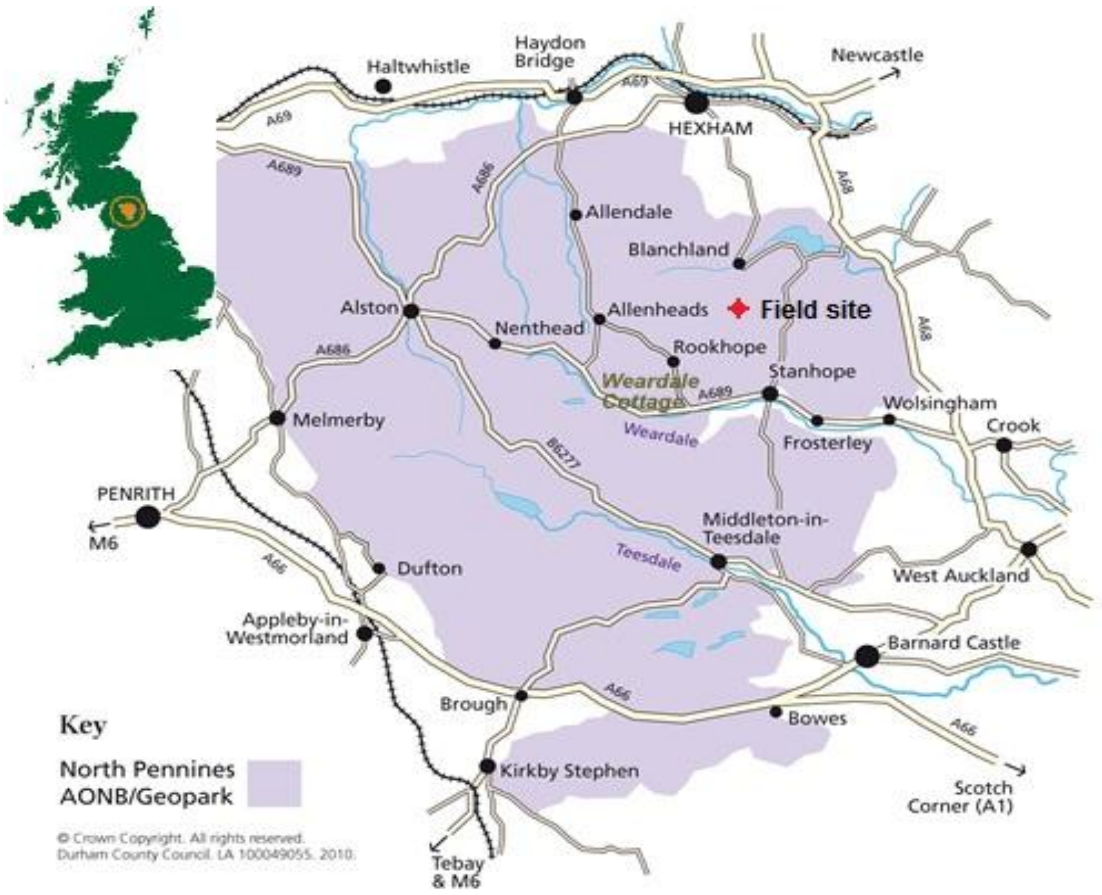

Figure 1: The Location of the UK field site (Durham County Council, 2010).

The Canadian site was located at Trail Valley Creek, approximately 60 km north of Inuvik, North West Territories (66° 44' 17" N 133° 26' 26" W) (Figure 2). The landscape is a mixture of low shrub and open tundra dominated by grasses, moss, and lichen. The site is within the zone of continuous permafrost and has a sub-arctic climate with an 8 month snow-cover period. The average maximum temperature for July is 19 °C, and the average minimum in January is -31 °C (University of Saskatchewan, 2014).

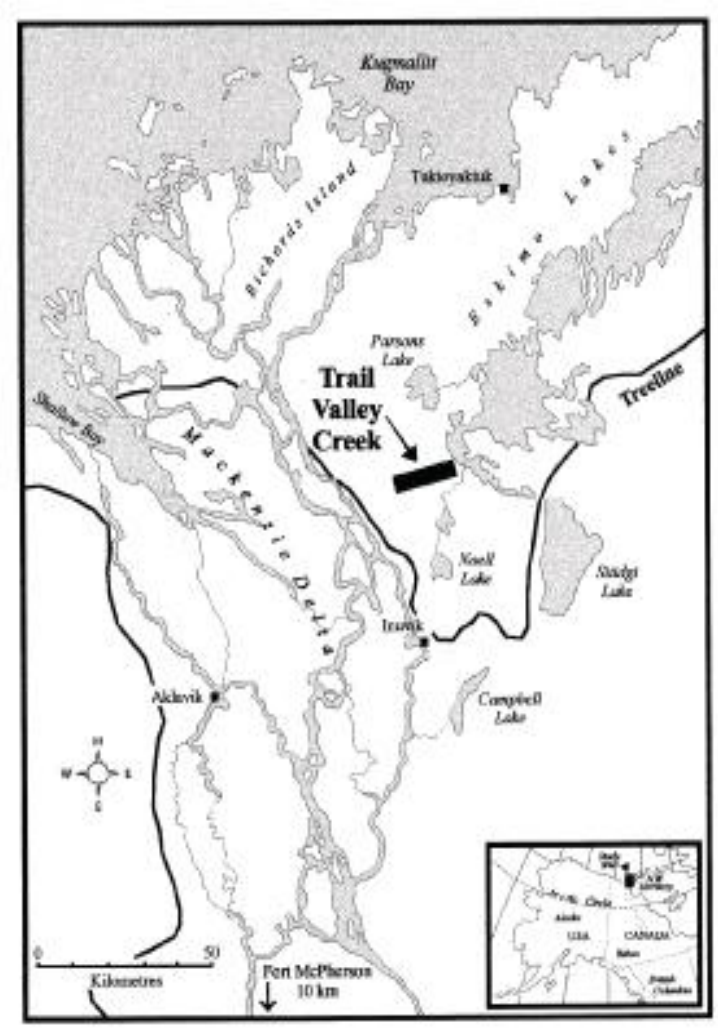

Figure 2: The location of the Canadian field site (University of Saskatchewan, 2014).

Study species

In total five different moss species were used in this study. Species were chosen to be representative of the landscape, both in their abundance and in their range of physiological traits. Nomenclature of mosses follows *Mosses and Liverworts of Britain and Ireland: a field guide* (British Bryological Society, 2010).

Hylocomium splendens has a widespread distribution in the northern hemisphere, and is abundant in both Europe and Canada. Of the five study species it was the only one studied at both UK and Canadian sites. *H. splendens* is a pleurocarpic 'feather' moss, with branching shoots up to 20 cm long. New shoots grow each year from the centre of the previous year's branch, resulting in a series of connected 'fronds'. *H. splendens* is commonly associated with woodland ecosystems but is also found on open moorland and arctic-alpine tundra.

Aulacomnium palustre is also widespread in the northern hemisphere, having a similar distribution to *H. splendens*. Whilst *A. palustre* was present at both sites it was only studied at the UK site. *A. palustre* commonly grows in wetland communities, and its presence is generally an indicator of wet soils. It is an acrocarpous moss which forms dense tufts of individual shoots with small, pointed leaves. Whilst shoots of this species can grow up to 10 cm tall, the samples taken from the UK field site rarely exceeded 2 cm in height.

Aulacomnium turgidum is similar in appearance to *A. palustre* but can be differentiated by the blunt, swollen appearance of its shoots and leaves. *A. turgidum* is common in cold environments, including the arctic and sub-arctic tundra regions. It was present only at the Canadian site, where it was more abundant than the related *A. palustre*. In the UK its distribution is limited to small areas of the Scottish highlands.

Polytrichum juniperinum is globally widespread and commonly found in dry and exposed habitats. It was present at both sites, but was not studied at the Canadian site as it was relatively scarce. *P. juniperinum* is an acrocarpous moss which forms open patches of erect, un-branched shoots approximately 4 cm tall. The stems have

thin, pointed leaves up to 1 cm long. It is often a pioneer species of recently burned or disturbed ground including quarries and woodland paths, a factor which is likely to have contributed to its abundance at the UK site.

Tomentypnum nitens is found in boreal and sub-arctic environments globally, particularly in wetland communities dominated by grasses and sedges. In this study *T. nitens* was found only at the Canadian site. This species is in decline in the UK, but can be found in upland mires, primarily in Scotland and Ireland. *T. nitens* is a pleurocarpic moss with upright stems up to 10 cm tall which form a dense carpet.

Part1: The UK

Collection and storage of samples

Samples of bryophyte material were collected from the UK field site in the North Pennine hills for use in the laboratory experiments. All the material used in experiments 1, 2 and 3 was collected in November 2013. Additional material was collected for chlorophyll analysis (experiment 4) in March and July 2014. Samples were collected and stored as complete turfs, including small quantities of non-study species, to minimise long-term damage and disturbance effects.

Turfs were placed in seed trays of dimensions 37 x 24 cm within a controlled environment chamber (Weiss Gallenkamp, Loughborough, UK) at 5 ˚C on an 8/16 h. day/night cycle and sprayed with water as necessary to maintain fully moist conditions (Figure 3). This environmental cycle was chosen to approximate autumn/winter conditions at the collection site, without the confounding effects of severe weather or freezing events.

Unfortunately, due to limited space inside the environmental chamber, the quantity of bryophyte material collected in the initial November survey was not enough to allow separate replicate moss turfs to be used in all the experiments which were subsequently developed. Rather than introduce confounding errors by mixing material collected later in the year, pseudo-replicate samples were taken from a single turf in each experimental condition. Individual shoots of moss were removed

from the turfs when required for experimentation, leaving the remainder as undisturbed as possible.

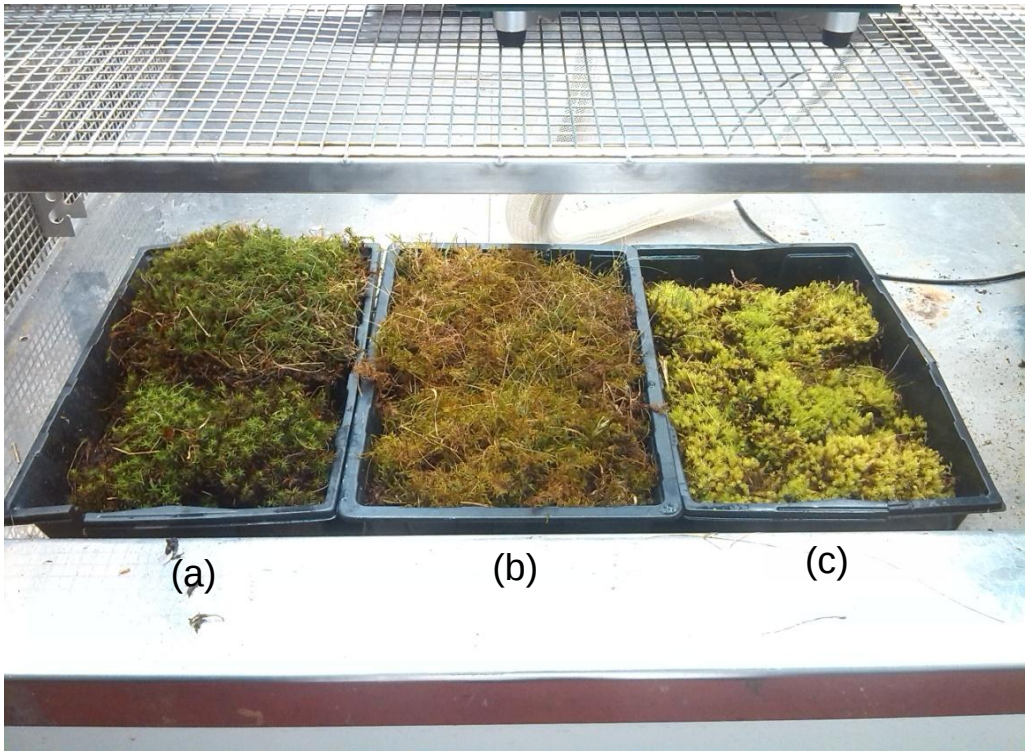

Figure 3: Moss species collected from the UK field site. (a) *Polytrichum juniperinum*, (b) *Hylocomium splendens*, and (c) *Aulacomnium palustre*.

Measurement of gas exchange in the laboratory

Gas exchange was measured using the LI-6400 portable photosynthesis system (LiCor, Lincoln, Nebraska, USA) in conjunction with the LI-COR bryophyte chamber. As the bryophyte chamber is a newly available piece of equipment it was first necessary to develop a reliable experimental protocol for its use. Due to the differing growth forms of the species used in this study, two different protocols were developed. The first was used with *P. juniperinum* and *H. splendens*, and the second with *A. palustre*. The following procedures were used to measure gas exchange in response to temperature change, water content, and freezing-thaw stress:

a) *Polytrichum juniperinum* and *Hylocomium splendens*

Individual shoots of moss were cut from the turfs and rinsed in distilled water. Due to limited space inside the bryophyte gas exchange chamber, the shoots were

trimmed to an approximate length of 2 cm. This corresponded to the most recent season's growth, with older material removed. The prepared shoots were blotted dry to remove excess water and placed inside the chamber. A small ring of black card, 3.5 cm in diameter and 2 cm in height, was used to hold the loose shoots in position. This ring also acted to reduce light penetration from the side, imitating the natural conditions of a continuous moss carpet with illumination from above. The shoots were arranged inside the chamber in such a way as to approximate the natural growth orientation and pattern of the species (Figure 4). An average of 0.3 g of *P. juniperinum* and 0.2 g of *H. splendens* was used in each experimental sample.

The complete photosynthesis system with the bryophyte chamber attached was placed inside a climate-controlled chamber (Weiss Gallenkamp Fitotron, Loughborough, UK). This chamber allowed the system to be cooled as necessary to the temperatures required for each experiment; where necessary fine-tuning of the bryophyte chamber temperature was done using the in-built LI-COR temperature control system (peltier module). Gas exchange was measured and subsequently calculated on a dry mass basis at 400 μmol mol^{-1} CO_2. Light intensity within the bryophyte gas exchange chamber was controlled using the fluorescent lights inside the growth chamber with a maximum PAR of 415 μmol m^{-2} s^{-1}. For measurements taken at 22 ˚C within the laboratory, light intensity was provided by an LED lamp array (Hansatech Ltd, King's Lynn, UK) suspended directly above the bryophyte chamber. This allowed a higher maximum light intensity of 675 μmol m^{-2} s^{-1}. A water jacket placed between the lamp and the chamber prevented the LEDs from having a significant warming effect on the bryophyte chamber. Chamber air humidity was maintained at *ca.* 70 % using the LI-COR humidity control system.

The rate of gas exchange was recorded at fifteen second intervals for ten minutes beginning immediately after each sample was placed inside the bryophyte chamber. Preliminary experiments showed that during this time the gas exchange rate typically increased as the moss acclimated to the ambient conditions, then decreased as the sample dried out (Preliminary results: Figure 8). Because the rate of gas exchange did not remain stable for more than a couple of minutes it was necessary to use a different sample of moss for each data point collected. To ensure

that measurements were consistent, the value recorded as the rate of gas exchange for each sample was the maximum rate measured over this ten-minute period.

b) *Aulacomnium palustre*

A. palustre naturally grows as a dense cushion which is small enough for complete sections to be placed inside the bryophyte chamber. These samples contain a significant quantity of dead tissue, with only the top *ca.* 5 mm of each shoot photosynthetically active. Preliminary experiments found that due to the high water content of these turfs, measurements could not be made without condensation forming inside the bryophyte chamber, risking damage to the equipment. To combat this, the turfs were separated into individual shoots which were rinsed in distilled water and trimmed where necessary to remove soil and non-focal species. The shoots were then blotted dry before being carefully placed back together. The same black card ring previously described was used to hold this artificial cushion together inside the bryophyte chamber. Each experimental sample contained *ca.* 3 g of tissue.

Preliminary experiments showed that this cushion dried much more slowly inside the chamber than the *P. juniperinum* and *H. splendens* samples, maintaining a steady rate of gas exchange 90 minutes or more (Preliminary results: Figure 9). Instead of using a different sample of tissue for each data point, the same sample could therefore be used to complete a full light response curve. The sample was placed inside the chamber and allowed to stabilise for fifteen minutes at the highest light intensity. The light intensity was then lowered every five minutes; with the rate of gas exchange measured at 60 s intervals during this period (Figure 9). The rate of net photosynthesis recorded was the last of the five measurements taken at each light level. This ensured that the plant had sufficient time to respond to the changing light intensity at each step.

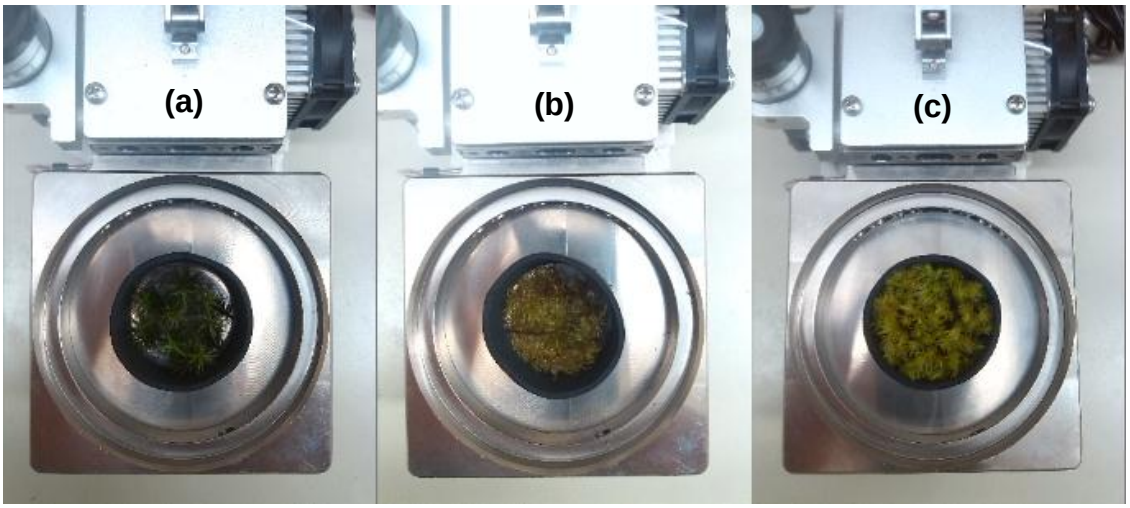

Figure 4: (a) *Polytrichum juniperinum*, (b) *Hylocomium splendens*, and (c) *Aulacomnium palustre* inside the small bryophyte chamber used for laboratory experiments.

Experimental design

Experiment 1: The effect of changing temperature on the photosynthetic activity of *Polytrichum juniperinum, Hylocomium splendens, and Aulacomnium palustre*

The first experiment was designed to investigate the effect of temperature on gas exchange, addressing hypothesis 1. Light response curves were produced at ambient temperatures of 1 ˚C, 5 ˚C, 10 ˚C and 22 ˚C for *P. juniperinum*, *H. splendens* and *A. palustre* using the above methods. Due to the dense nature of the *A. palustre* cushion these samples retained heat, raising the temperature of the moss cushion to 1 ˚C warmer than ambient at cooler temperatures. The data are therefore presented as being recorded at 2 ˚C and 6 ˚C instead of 1 ˚C and 5 ˚C for this species. Five replicate light response curves were produced for each species at each temperature. Measurements of gas exchange are presented per gram of dry mass.

Experiment 2: The effect of changing bryophyte water content on the photosynthetic activity of *Polytrichum juniperinum* and *Hylocomium splendens*

The second experiment was designed to investigate the effect of water content on gas exchange, addressing hypothesis 1. 24 fully hydrated samples of *P. juniperinum*

and 26 of *H. splendens* were prepared as previously described. Each sample was then allowed to dry naturally for between 0 and 60 minutes to produce samples with a range of different water contents. Samples were weighed immediately before net photosynthesis was measured at 675 μmol m^{-2} s^{-1} PAR, 400 μmol mol^{-1} CO_2, and at an ambient temperature of 22˚C. Samples were then dried at 80˚C for 24h and weighed again to calculate the water content of each sample at the time of gas exchange determination.

Experiment 3: The effect of freeze-thaw conditions on the photosynthetic activity of *Polytrichum juniperinum* and *Hylocomium splendens*

The third experiment was designed to investigate the effect of hydration status during freeze-thaw cycles on the long-term gas exchange capability of mosses, addressing hypothesis 2. Complete turfs of *P. juniperinum* and *H. splendens* were moved to a climate-controlled chamber at 1 ˚C for two weeks, during which time they were kept fully hydrated. The chamber temperature was then gradually increased to 10 ˚C at a rate of 2 ˚C per hour, after which it was maintained on a 10 °C/5 ˚C day/night cycle for a total of seven days.

During the warming period each turf was split into two pieces which became the hydrated and dehydrated conditions for that species. The hydrated condition turfs were sprayed with water regularly to retain a moist environment, whilst the dehydrated turfs were allowed to become air-dry through natural evaporation and air movement within the climate-controlled chamber during this period.

After seven days the chamber temperature was lowered to -10 ˚C, again at a rate of 2 ˚C per hour. It was then maintained on a -5 °C/-10 ˚C day/night cycle for a further seven days. After this time the temperature was once again raised to 10˚C at a rate of 2˚C per hour. Once thawed, both the hydrated and dehydrated turfs were returned to full hydration. The chamber was then kept at a constant 10 ˚C for the duration of the 12-day post-freezing period.

Net photosynthesis was measured in four replicate sub-samples of material collected from each turf at four points during the experiment. Measurements were

taken: (1) before freezing, and (2) 24h, (3) 5 days and (4) 12 days after thawing. Photosynthesis was measured at full hydration, 10°C, 400 µmol mol^{-1} CO_2, and 415 µmol m^{-2} s^{-1} PAR.

Experiment 4: Seasonal variation in the chlorophyll content of *Polytrichum juniperinum, Hylocomium splendens, and Aulacomnium palustre*

This experiment was designed to measure seasonal variation in the chlorophyll content of the study species *in situ*. Chlorophyll content is highly correlated with photosynthetic capacity, and low leaf chlorophyll concentration can indicate cellular damage due to freezing and other naturally occurring stresses. This experiment therefore addresses both hypotheses 1 and 2.

Samples of *P. juniperinum*, *H. splendens* and *A. palustre* were collected in November 2013, March 2014 and July 2014. Chlorophyll content was measured in six replicate sub-samples of each species at each time point. Shoots of all three species were trimmed so that only <1 year old green tissue was used in this experiment. The samples were rinsed with distilled water and blotted dry before being weighed. Because the chlorophyll extraction process destroys the samples it was not possible to obtain an accurate dry weight measurement. The results are therefore presented on a fresh weight basis. For each sample the chlorophyll was extracted according to the following method:

0.2 g of hydrated moss was ground in a 4 ml of ice-cold 96 % ethanol, using a pinch of acid-washed silver sand as an aid to disintegration. The solution was added to an ice-cold centrifuge tube, and the pestle and mortar rinsed with an additional 4 ml of ice-cold ethanol which was also added. The extract was then centrifuged at 3000 g for 5 minutes. The supernatant was decanted into a 10 cm^3 volumetric flask and made up to volume with additional ice-cold 96 % ethanol. The samples were kept in darkness to prevent degradation.

To determine the chlorophyll content the optical density of the sample was measured using a spectrophotometer (UV-150-02, Shimadzu, UK) at 649, 665, and 470 nm wavelengths of light. The total concentration of chlorophyll a, chlorophyll b,

and carotenoids in each sample (μg cm^{-3} extract) was then determined using the following calculations (Arnon, 1949):

Chlorophyll a = 13.95 x A_{665} – 6.88 x A_{649}

Chlorophyll b = 24.96 x A_{649} – 7.32 x A_{665}

Carotenoids = (1000 x A_{470} – 2.05 x C_a – 114.8 x C_b) / 245

The results were then converted to μg g^{-1} fresh weight.

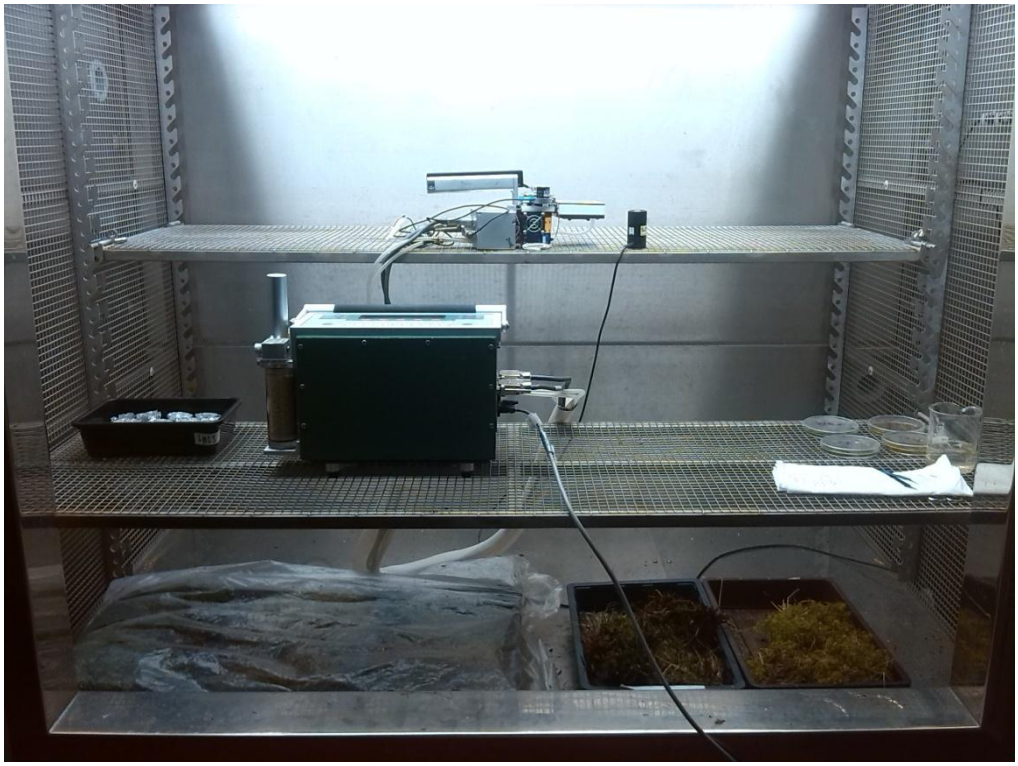

Figure 5: LI-COR photosynthesis system set up with bryophyte chamber inside the climate controlled chamber.

Experiment 5: Spring temperature variation of the moss carpet

To provide contextual temperature data for these experiments a temperature data logger (Tiny Tag plus 2, Gemini data loggers Ltd, Chichester, UK) was placed at the UK field site. The logger was placed directly on the moss carpet beneath a patch of heather, thereby recording unshielded moss surface temperature. The maximum and minimum temperature of the moss surface was recorded daily throughout late winter and spring between the 21st of January and the 8th of July 2014.

Part 2: Canada

Collection of samples

Field measurements of whole plant community-level gas exchange were conducted at the field site in Canada in June 2014. The site was surveyed and patches of ground containing a majority (>90 %) of one of the three focal study species (*H. splendens, A. turgidum and T. nitens*) were selected for sampling. For each species three circular turfs with a diameter of 15 cm and a height of 6 cm were removed and placed inside an opaque plastic collar of the same size. During the early season the permafrost layer is close to the surface in this area of the arctic. There was therefore a clear distinction between the living moss layer and the frozen soil beneath allowing the turfs to be removed with ease. Turfs were kept largely intact with only obvious vascular plant growth carefully removed. Excess water was removed by gentle blotting to prevent condensation inside the chamber.

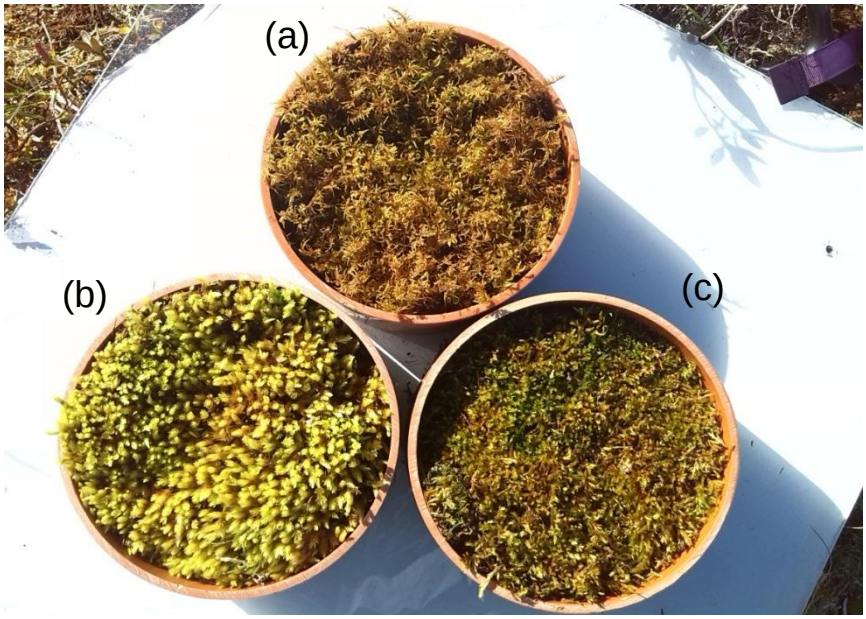

Figure 6: Moss species collected from the Canada field site. (a) *Hylocomium splendens*, (b) *Aulacomnium turgidum*, (c) *Tomentypnum nitens*.

Measurement of gas exchange in the field

Gas exchange was measured using the same LI-6400 photosynthesis system used in the laboratory experiments. The small bryophyte chamber was removed and a clear

open-bottomed acrylic chamber measuring 30 x 30 x 20 cm was attached to the IRGA (Figure 7). Each collar containing a moss sample was placed separately on an acrylic sheet, with the clear chamber placed over the top to create an airtight seal. No artificial light source was used in the field experiments; instead a light sensor inside the chamber (LiCOR LI-190 Quantum Sensor, LiCOR Ltd, Lincoln Nebraska, USA) recorded the ambient light level. Experiments were conducted at ambient CO_2 concentration and air temperature. A small fan inside the chamber ensured sufficient breakdown of the vegetation boundary layer resistance to allow accurate readings of CO_2 flux.

The change in CO_2 concentration inside the chamber was measured over a 40 s period, beginning as soon as the chamber was closed with the sample inside. These data were then used to calculate the average CO_2 flux during the 40 s period using the following calculation from Street et al., (2007).

$$F_c = (\rho \times V \times dC/dt) / A$$

Where F_c is net CO_2 flux (μmol m^{-2} s^{-1}), ρ is air density (mol m^{-3}), V is the chamber CO_2 volume (m^3), dC/dt is the slope of chamber CO_2 concentration against time (μmol mol^{-1} s^{-1}) and A is the chamber surface area (m^2).

These results were calculated on a unit surface-area basis and are therefore not directly comparable to the results produced by the earlier laboratory experiments. Light response curves were produced by covering the chamber in layers of optically neutral cloth which reduced light penetration inside the chamber. Two or three measurements were taken with each sample at each shade level. As the ambient light level varied naturally throughout the experimental period, these readings were not exact replicates, but provided a wider spread of data points on the final curves.

Experimental design

Experiment 6: Field measurements of whole-plant community-level gas exchange in *Hylocomium splendens, Aulacomnium turgidum* and *Tomentypnum nitens*

A light response curve was produced using the data collected from each of the three replicate turfs of each species under similar weather conditions (12-13 °C sun/cloud). This process was then repeated using the same sample turfs after they had been left to dry naturally for 24 h under the same weather conditions. The temperature of the moss turf was recorded at a depth of 1 cm during each measurement period for later comparison. An additional set of measurements was taken for *H. splendens* under colder conditions (6 °C) but unfortunately, due to equipment problems, this was not repeated with the other species. Immediately after each set of gas exchange measurements was taken, a small sub-sample of moss was removed from the turf and its water content determined gravimetrically.

Figure 7: *Hylocomium splendens* inside the large chamber used for field experiments.

Data analysis

Light response curves from both the laboratory and field experiments were fitted using proprietary software (PHOTOSYN Assistant, Dundee Scientific, Dundee, UK). The program was used to calculate values of the maximum net photosynthetic rate (A_{max}), light compensation point, light saturation point, and quantum efficiency (QE) from each curve. The data from the laboratory experiments were tested for normality, and then a series of one-way ANOVA tests were used to compare these values from data recorded at different temperatures for each species. The data from the field experiments were compared using a series of paired t-tests.

The relationship between sample water content and net photosynthetic rate was determined by plotting all the data points for each species on a scatter graph and conducting a linear regression analysis.

Freeze-thaw data were analysed by calculating the mean net photosynthetic rate for both hydrated and dehydrated conditions at each time point during the experiment. The data were then tested for normality and the values compared using a two way ANOVA for each species.

The mean chlorophyll content, chlorophyll a:b ratio, and carotenoid content of each moss species in November, March and July was also compared using a series of one-way ANOVA tests after testing for normality. All statistical computations were done using statistical software (SPSS Statistics for Windows, Version 20.0).

Chapter Three: Results

Preliminary experiments – development of methodology

When samples of *P. juniperinum* and *H. splendens* were placed inside the bryophyte chamber, the rate of gas exchange reached a maximum after *ca.* 4 minutes, after which it declined due to water loss from the sample (Figure 8). In contrast, the rate of gas exchange of *A. palustre* was very stable, reaching its maximum after *ca.* 15 minutes and remaining constant for a further 100 minutes before beginning to fall (Figure 9). These results were used to determine the best way to measure the gas exchange rate for each species, and to develop the two different protocols used in the study. Due to the differences in the experimental protocol used with *A. palustre* compared to the other species, it is not possible to directly compare these results to those from *P. juniperinum* and *H. splendens*. In the interest of clarity they have therefore been presented separately.

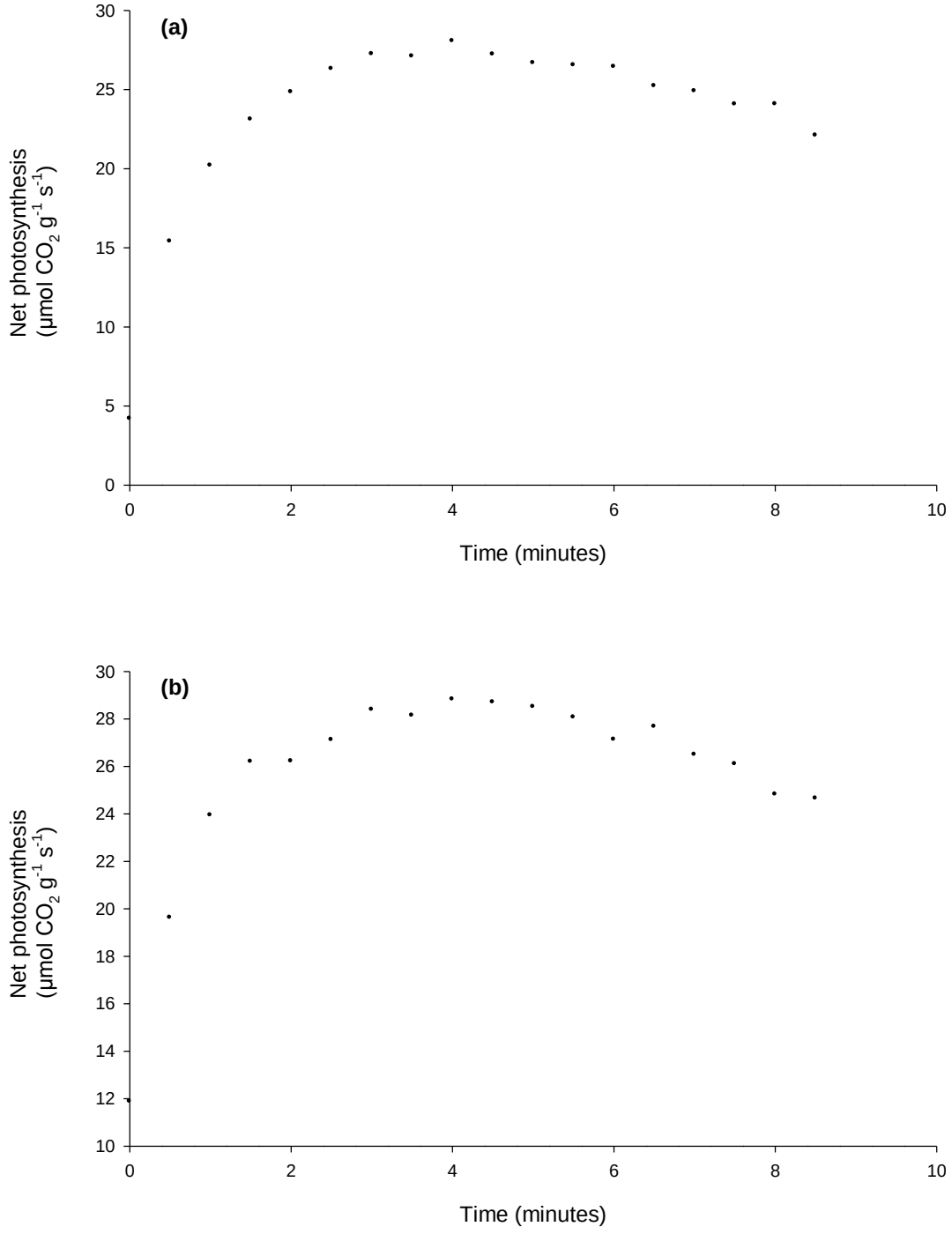

Figure 8: The rate of gas exchange of *Polytrichum juniperinum* (a), and *Hylocomium splendens* (b) inside the bryophyte chamber.

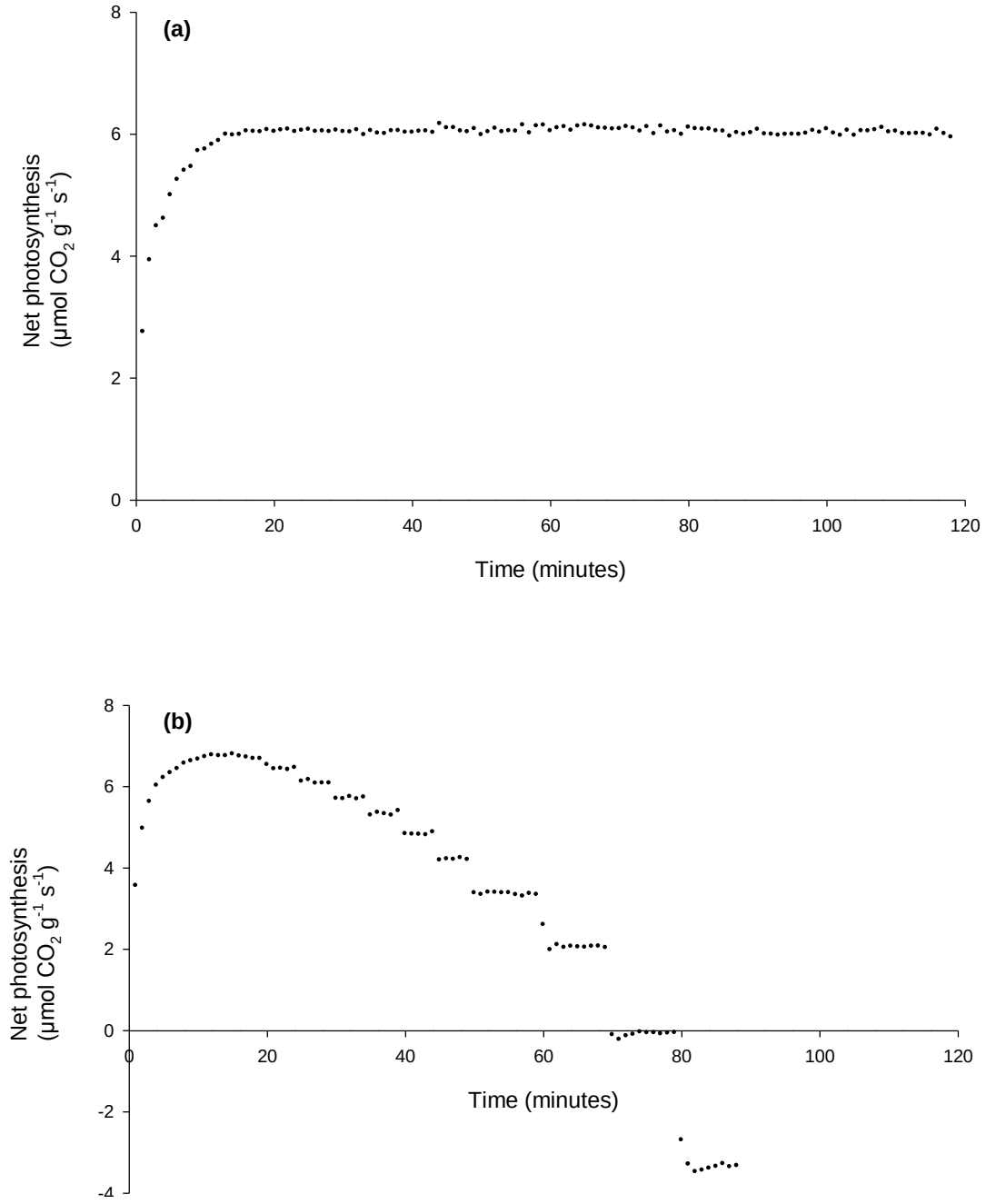

Figure 9: The rate of gas exchange of *Aulacomnium palustre* under stable light intensity (a) and changing light intensity (b) inside the bryophyte chamber.

Experiment 1a: The effect of changing temperature on the photosynthetic activity of *Polytrichum juniperinum* and *Hylocomium splendens*.

The aim of this experiment was to determine the optimum temperature for moss growth by measuring the gas exchange capability of the samples at a range of temperatures, and producing light response curves to compare key photosynthetic traits.

Maximum net photosynthesis

The maximum net photosynthetic rate (A_{max}) of both *P. juniperinum* and *H. splendens* increased significantly with temperature (Figure 10, Table 1). In *P. juniperinum* A_{max} more than doubled (an increase of 115%) between 1 °C and 22 °C ($p<0.001$). However there was no significant increase between 1 °C and 5 °C, or between 10 °C and 22 °C (Figure 10). A_{max} also more than doubled (an increase of 137%) between 1 °C and 22 °C in *H. splendens* ($p<0.001$). In *H. splendens* there was a significant increase in A_{max} with each increase in temperature step measured in the study (Figure 10). The percentage increase in A_{max} with each 1 °C increase in temperature was higher at lower temperatures. Between 1 °C and 5 °C there was a *ca.* 10% increase in A_{max} with every 1 °C rise in temperature. Between 5 °C and 10 °C this increase was *ca.* 7.5%, and between 10 °C and 22 °C the increase was only *ca.* 1.2% with each 1 °C increase in temperature.

Dark respiration

The dark respiration rate of both *P. juniperinum* and *H. splendens* increased significantly with temperature (Figure 10, Table 1). In *P. juniperinum* the dark respiration rate rose by a factor of 5 between 1 °C and 22 °C, giving an average Q_{10} value of 2.51. The dark respiration rate measured at 1 °C in *H. splendens* was very low (0.09 μmol CO_2 g^{-1} s^{-1} compared to 0.92 μmol CO_2 g^{-1} s^{-1} in *P. juniperinum*), resulting in a ~50 fold increase in respiration rate between 1 °C and 22 °C, and giving an average Q_{10} value of 26.5. If the measurement at 1 °C is discounted as an anomaly, the average Q_{10} value between 5 °C and 22 °C is 1.82.

Quantum efficiency

The quantum efficiency of *P. juniperinum* showed no significant change in response to temperature (Fig 11; Table 1). The average quantum efficiency of *P. juniperinum* was estimated to be 0.26%. In *H. splendens* there was a significant increase in quantum efficiency with temperature (Fig 11, $p<0.001$), rising from 0.14% at 1°C to 0.46% at 22°C.

Light compensation point

The light compensation point of both *P. juniperinum* and *H. splendens* increased significantly with temperature (Figure 11; Table 1). In *P. juniperinum* the light compensation point increased from 3.5 to 17 μmol m^{-2} s^{-1} between 1 °C and 22°C ($p<0.001$). In *H. splendens* it increased from 1.3 to 10 μmol m^{-2} s^{-1} between 1 °C and 22°C ($p<0.001$).

Light saturation point

In *P. juniperinum* there was a significant increase in the light saturation point with temperature (Figure 11, Table 1). Between 1 °C and 22 °C the light saturation point increased from 90.6 to 170 μmol m^{-2} s^{-1}. This increase followed the same pattern seen in the response of the net photosynthetic rate, with no significant increase between 1 °C and 5 °C or between 10 °C and 22 °C (Figure 11). The light saturation point of *H. splendens* showed no significant change in response to temperature, and maintained an average value of 120 μmol m^{-2} s^{-1}.

Table 1: One-way ANOVA analysis of the results of experiment 1: The effect of changing temperature on bryophyte photosynthetic activity. Significance indicators represent: not significant ($p>0.05$) * ($p<0.05$) ** ($p<0.01$) *** ($p<0.001$).

Species	Value	ANOVA				
		df		F	p	Sig.
		Within	Between			
P. juniperinum	A_{max}	17	3	53.6	<0.001	***
	QE	17	3	0.8	0.51	ns
	Light comp. point	17	3	20.7	<0.001	***
	Light sat. point	17	3	15.1	<0.001	***
	Respiration	17	3	18.5	<0.001	***
H. splendens	A_{max}	16	3	67	<0.001	***
	QE	16	3	19.1	<0.001	***
	Light comp. point	16	3	9.2	0.001	***
	Light sat. point	16	3	1.7	0.21	ns
	Respiration	16	3	13.1	<0.001	***
A. palustre	A_{max}	13	3	18.7	<0.001	***
	QE	13	3	1.08	0.39	ns
	Light comp	13	3	72.2	<0.001	***
	Light sat	13	3	9.69	0.001	***
	Respiration	13	3	42.8	<0.001	***

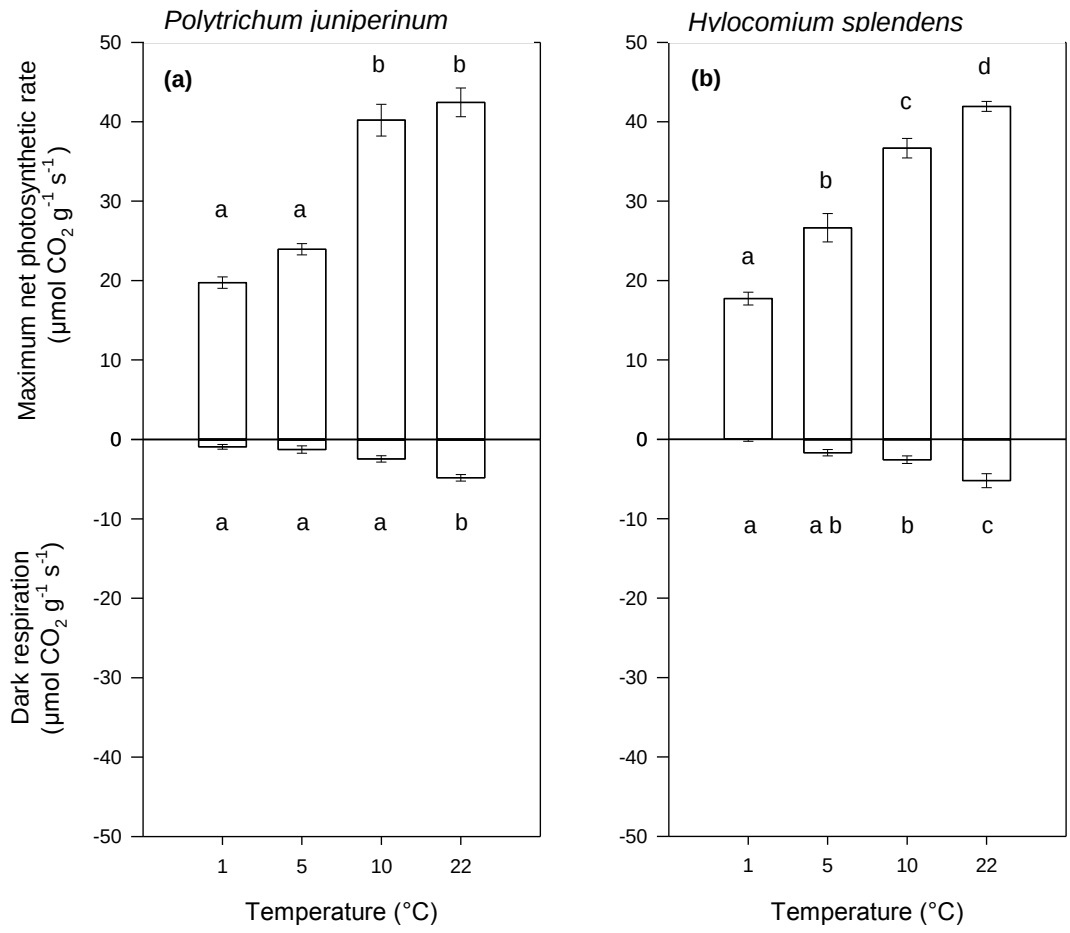

Figure 10: Mean values of maximum net photosynthesis and dark respiration calculated from the analysis of light response curves of (a) *Polytrichum juniperinum* and (b) *Hylocomium splendens* at a range of ambient temperatures. Error bars represent +/- 1SEM. Different letters denote statistically significant differences at $p<0.05$.

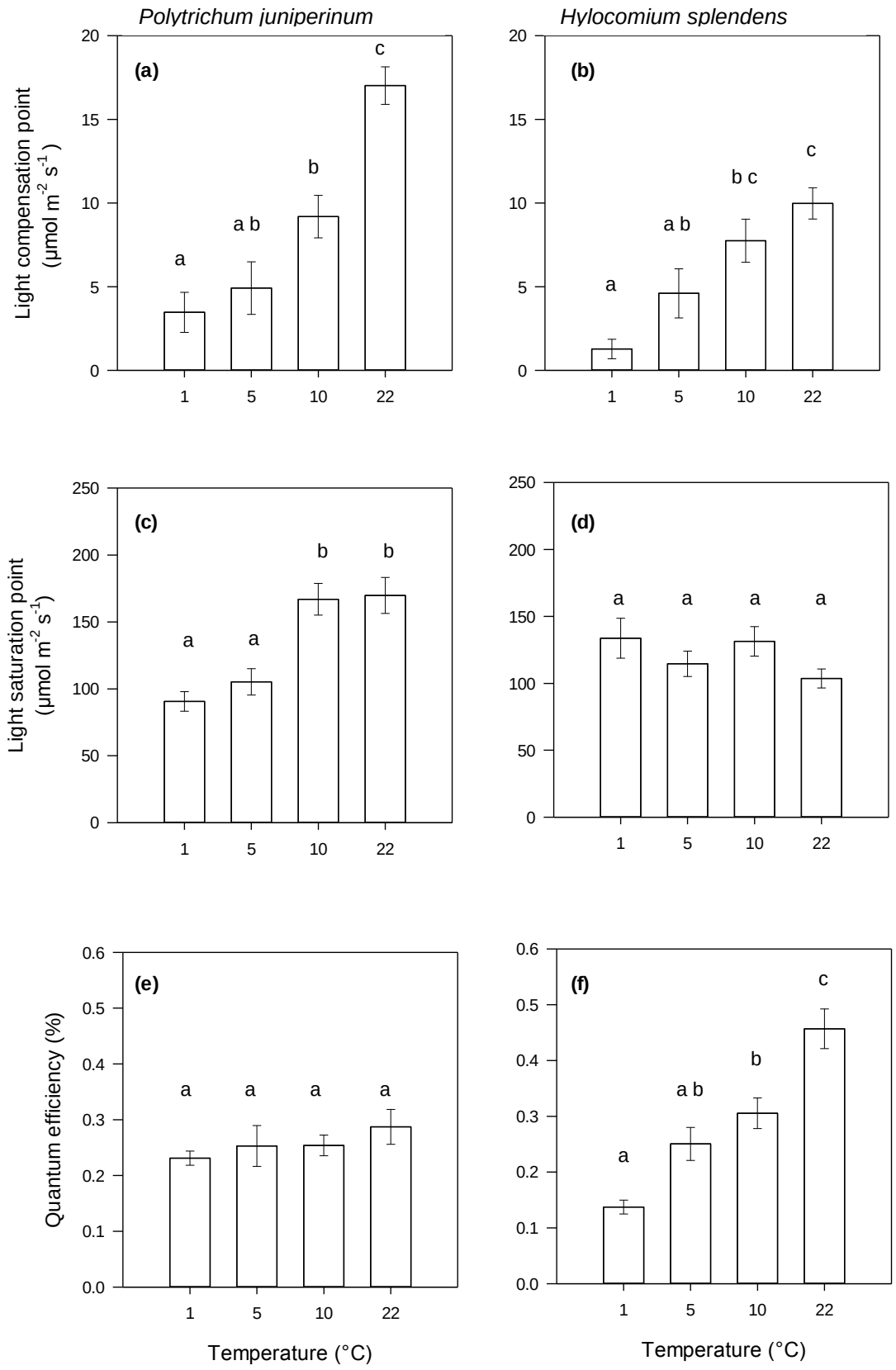

Figure 11: Mean values of Light Compensation point (a,b), Light Saturation point (c,d) and Quantum Efficiency (e,f) calculated from the analysis of light response curves of *Polytrichum juniperinum* and *Hylocomium splendens* at a range of ambient temperatures. Error bars represent +/- 1SEM. Different letters denote statistically significant differences at $p<0.05$.

Experiment 1b: The effect of changing temperature on the photosynthetic activity of *Aulacomnium palustre*.

Maximum net photosynthesis

The maximum net photosynthetic rate of *A. palustre* increased significantly with temperature (Figure 12, Table 1). Between 2 °C and 22 °C there was a 30% increase in A_{max} ($p<0.001$). As in *P. juniperinum*, there was no significant change in the photosynthetic rate of *A. palustre* between 10 °C and 22 °C.

Dark respiration

The dark respiration rate of *A. palustre* increased significantly with temperature (Figure 12, Table 1). Between 2 °C and 22 °C the respiration rate approximately tripled (an increase of 216%), giving an average Q_{10} value of 1.6.

Quantum efficiency

There was no significant change in quantum efficiency with temperature in *A. palustre* (Figure 13, Table 1). The average quantum efficiency of this species was estimated to be 0.08%.

Light compensation point

The light compensation point of *A. palustre* increased significantly with temperature (Figure 13; Table 1), increasing from 10.7 to 41.3 μmol m^{-2} s^{-1} between 2 °C and 22°C ($p<0.001$).

Light saturation point

The light saturation point of *A. palustre* also increased significantly with temperature (Figure 13, Table 1), increasing from 120 to 198 μmol m^{-2} s^{-1} between 2 °C and 22°C ($p=0.001$)

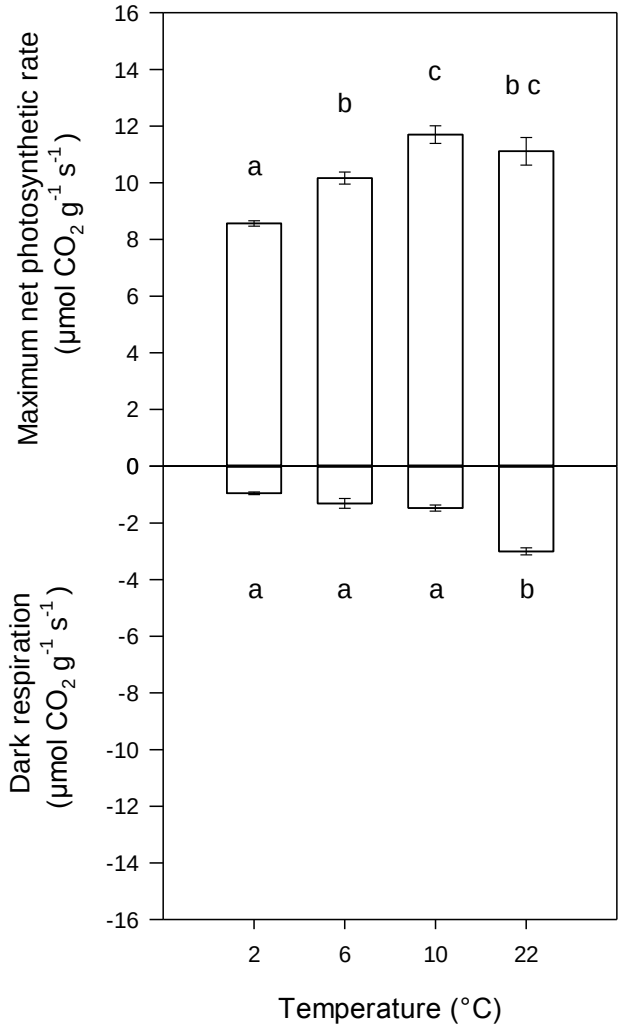

Figure 12: Mean values of maximum net photosynthesis and dark respiration calculated from the analysis of light response curves of *Aulacomnium palustre* at a range of ambient temperatures. Error bars represent +/- 1SEM. Different letters denote statistically significant differences at $p<0.05$.

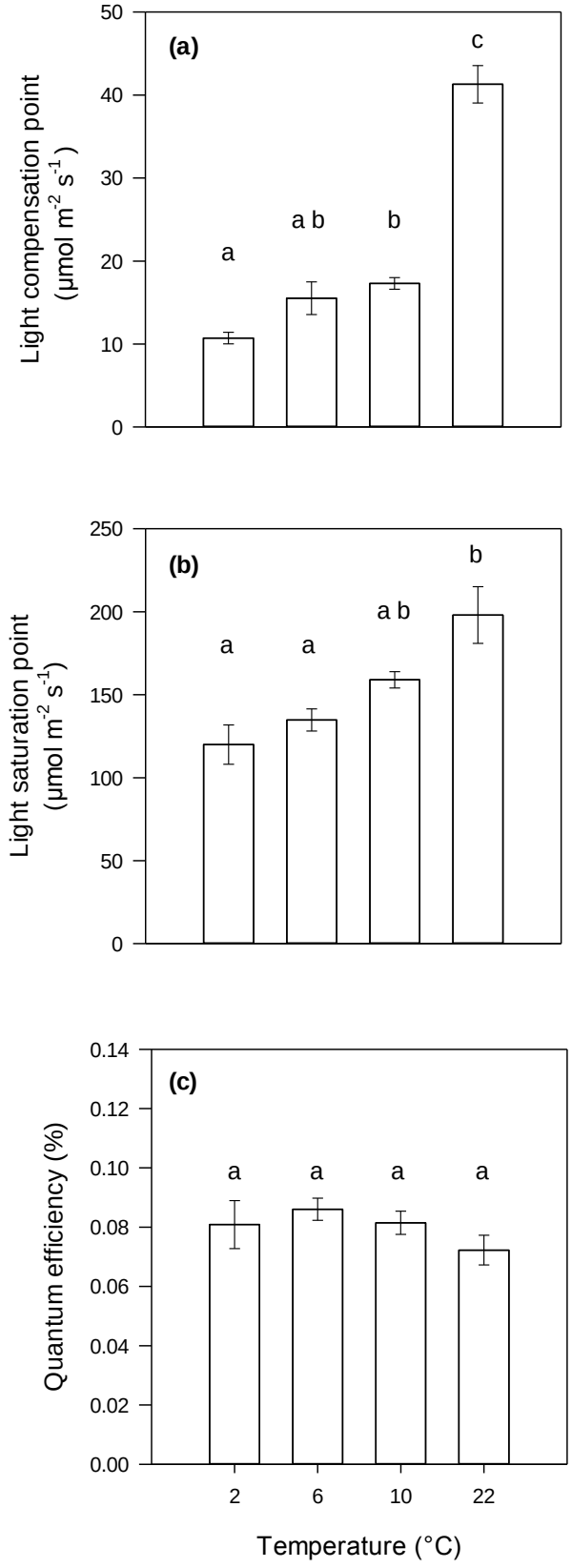

Figure 13: Mean values of (a) Light Compensation Point, (b) Light Saturation Point and (c) Quantum Efficiency, calculated from the analysis of light response curves of *Aulacomnium palustre* at a range of ambient temperatures. Error bars represent +/- 1SEM. Different letters denote statistically significant differences at $p<0.05$.

Experiment 2: The effect of changing water content on the photosynthetic activity of *Polytrichum juniperinum* and *Hylocomium splendens*

The net photosynthetic rate was strongly negatively correlated with moss water content in both *P. juniperinum* (Figure 14, Pearson correlation = 0.97, n=24, $p<0.001$) and *H. splendens* (Pearson correlation = 0.96, n=26, $p<0.001$). Linear regression analysis predicts that the moisture compensation point is approximately 71% in *P. juniperinum* and 46% in *H. splendens*. The maximum rate of net photosynthesis at 22°C was reached at approximately 180% water content in *P. juniperinum* and 230% in *H. splendens*. The slopes of the linear regression lines suggest that *P. juniperinum* is more sensitive to water loss than *H. splendens*. The net photosynthetic rate of *P. juniperinum* fell by 3.3 μmol CO_2 g^{-1} s^{-1} with each 10% drop in water content. In contrast the net fall in photosynthetic rate of *H. splendens* was approximately half this value, a fall of 1.8 μmol CO_2 g^{-1} s^{-1} with each 10% drop in water content.

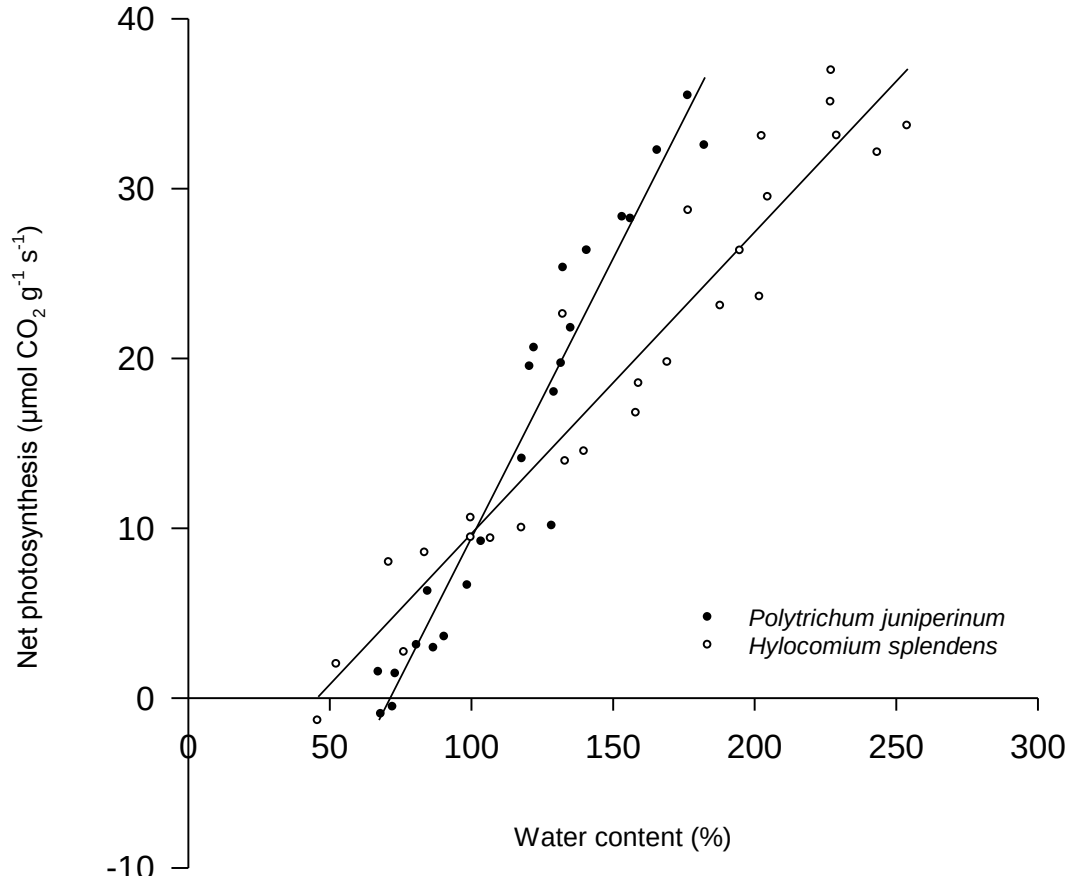

Figure 14: The net photosynthetic rate of samples of *Polytrichum juniperinum* and *Hylocomium splendens* in relation to their relative water content. Lines of best fit represent linear regression analyses of each data set. *P. juniperinum* $R^2 = 0.94$, *H. splendens* $R^2 = 0.93$.

Experiment 3: The effect of freeze-thaw conditions on the photosynthetic activity of *Polytrichum juniperinum* and *Hylocomium splendens*

The aim of this experiment was to measure the effect of a freezing event on bryophyte growth by comparing net photosynthesis before and after the event. The experiment also investigated whether the response differed according to the water content of the moss at the time of freezing.

Polytrichum juniperinum

Freezing was associated with a significant drop in the net photosynthetic rate of *P. juniperinum* (Fig 15; Table 2). 24 h after thawing, net photosynthesis of both hydrated and dehydrated samples was an average of 18% lower compared to pre-freezing measurements. The hydration status of the moss at the time of freezing also had a significant effect on the photosynthetic rate ($P<0.001$). In the dehydrated samples, net photosynthesis gradually increased after the 24 h measurement and after 12 days had recovered to pre-freezing levels. In the hydrated samples, net photosynthesis continued to fall after thawing. After five days net photosynthesis was an average of 31% lower in hydrated samples compared to dehydrated samples, and 38% lower than pre-freezing values. After 12 days net photosynthesis had begun to recover in the hydrated samples, but was still 18% lower than pre-freezing levels.

Hylocomium splendens

Freezing was also associated with a small, but significant, drop in the net photosynthetic rate of *H. splendens* (Figure 15, Table 2). Whilst there was no significant change after 24 h, by 5 days after thawing, net photosynthesis was an average of 13% lower than pre-freezing measurements, under both hydrated and dehydrated conditions. In *H. splendens*, there was no significant effect of hydration status at the time of freezing on the post-thaw photosynthetic rate ($p=0.75$) and 12 days after thawing both samples had recovered to pre-freezing rates of net photosynthesis.

Table 2: Two-way ANOVA analysis of the results of experiment 3: The effect of freeze-thaw conditions on bryophyte photosynthetic activity. Significance indicators represent: not significant ($p>0.05$) * ($p<0.05$) ** ($p<0.01$) *** ($p<0.001$).

Species	Factor	2-way ANOVA			
		df	F	p	Sig.
P. juniperinum	Time	3	27.4	<0.001	***
	Hydration status	1	31.4	<0.001	***
	Time * Hydration	3	12.3	<0.001	***
H. splendens	Time	3	4.39	0.013	*
	Hydration status	1	0.102	0.753	ns
	Time * Hydration	3	1.84	0.168	ns

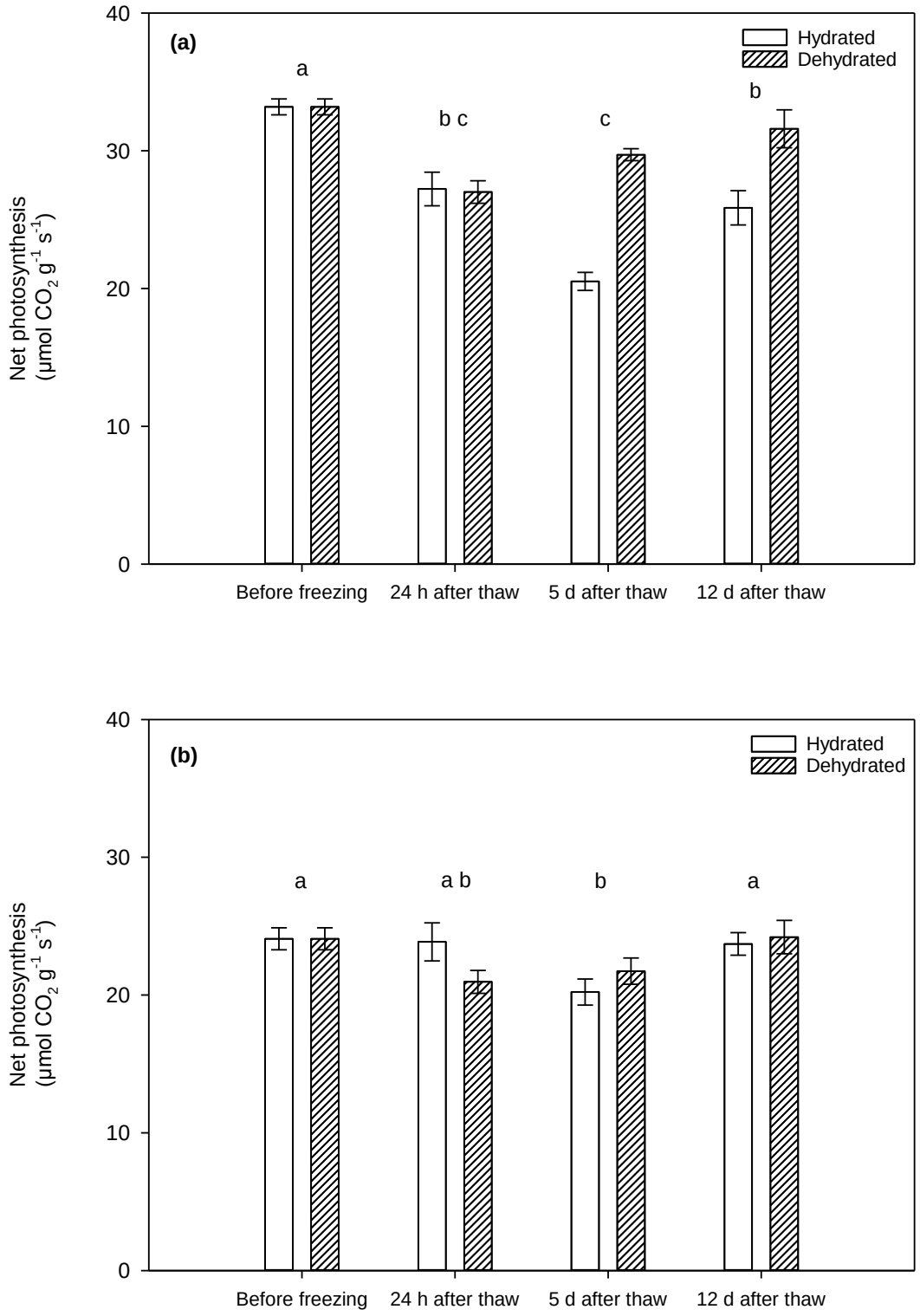

Figure 15: Rates of net photosynthesis before and after freezing stress in *Polytrichum juniperinum* (a), and *Hylocomium splendens* (b). Error bars represent +/- 1SEM. Letters denote statistically significant differences at the $p<0.05$ level.

Experiment 4: Seasonal variation in chlorophyll content in *Polytrichum juniperinum*, *Hylocomium splendens* and *Aulacomnium palustre*

The aim of this experiment was to determine the extent to which seasonal environmental stress affects the chlorophyll content of mosses *in situ.*

Total chlorophyll content

The total chlorophyll content of all three moss species varied significantly over the course of one year (Figure 16, Table 3). In both *H. splendens* and *A. palustre* there was a significant drop in plant chlorophyll content over the winter months, with values measured in March significantly lower than those measured in November ($p<0.001$). In *H. splendens*, total chlorophyll content fell by 46% over this period and in *A. palustre* it fell by 49%. There was no significant change in the chlorophyll content of *P. juniperinum* over the winter months. The chlorophyll content of *A. palustre* then increased by 28% during the spring between March and July. No significant change was seen over this period in *H. splendens*. In *P. juniperinum* there was a significant drop of 31% in total chlorophyll content between March and July ($p<0.001$).

Chlorophyll a:b ratio

The mean ratio of chlorophyll a:b measured in *P. juniperinum* was 2.18. This value did not show any significant seasonal variation (Figure 16). Seasonal variation in the chlorophyll a:b ratio was seen in *H. splendens* and *A. palustre* (Figure 16, Table 3). In *H. splendens* the ratio fell from 2.23 in November, a value comparable to that of *P. juniperinum,* to only 1.69 in March. This change reflects a much larger drop in the content of chlorophyll a in the plant over this period compared to chlorophyll b. As with the total chlorophyll content, this ratio did not change between March and July. The ratio of chlorophyll a:b in *A. palustre* did not change between November and March. Between March and July the ratio fell from 2.34 to 1.87, a period during which the total chlorophyll content increased. This change therefore reflects a greater increase in the chlorophyll b concentration over this period compared to chlorophyll a.

Carotenoid content

Seasonal change in the carotenoid content of these species mirrored the change in total chlorophyll concentration over the same period (Figure 16), showing significant seasonal variation in all three species (Table 3). The carotenoid concentration in *H. splendens* and *A. palustre* fell between November and March, by 49% and 38% respectively but showed no change between March and July. In *P. juniperinum* there was no change in carotenoid content between November and March, but there was a significant drop of 35% between March and July ($p<0.001$).

Table 3: One-way ANOVA analysis of the results of experiment 4: Seasonal variation in bryophyte chlorophyll content. Significance indicators represent: not significant ($p>0.05$) * ($p<0.05$) ** ($p<0.01$) *** ($p<0.001$).

Species	Value	ANOVA				
		df		F	p	Sig.
		Within	Between			
P. juniperinum	Total Chl	15	2	10.6	0.001	***
	Chl a:b ratio	15	2	0.9	0.43	ns
	Carotenoids	15	2	35.3	<0.001	***
H. splendens	Total Chl	15	2	87.9	<0.001	***
	Chl a:b ratio	15	2	6.87	0.008	**
	Carotenoids	15	2	45.7	<0.001	***
A. palustre	Total Chl	15	2	99.6	<0.001	***
	Chl a:b ratio	15	2	12.4	0.001	***
	Carotenoids	15	2	15.1	<0.001	***

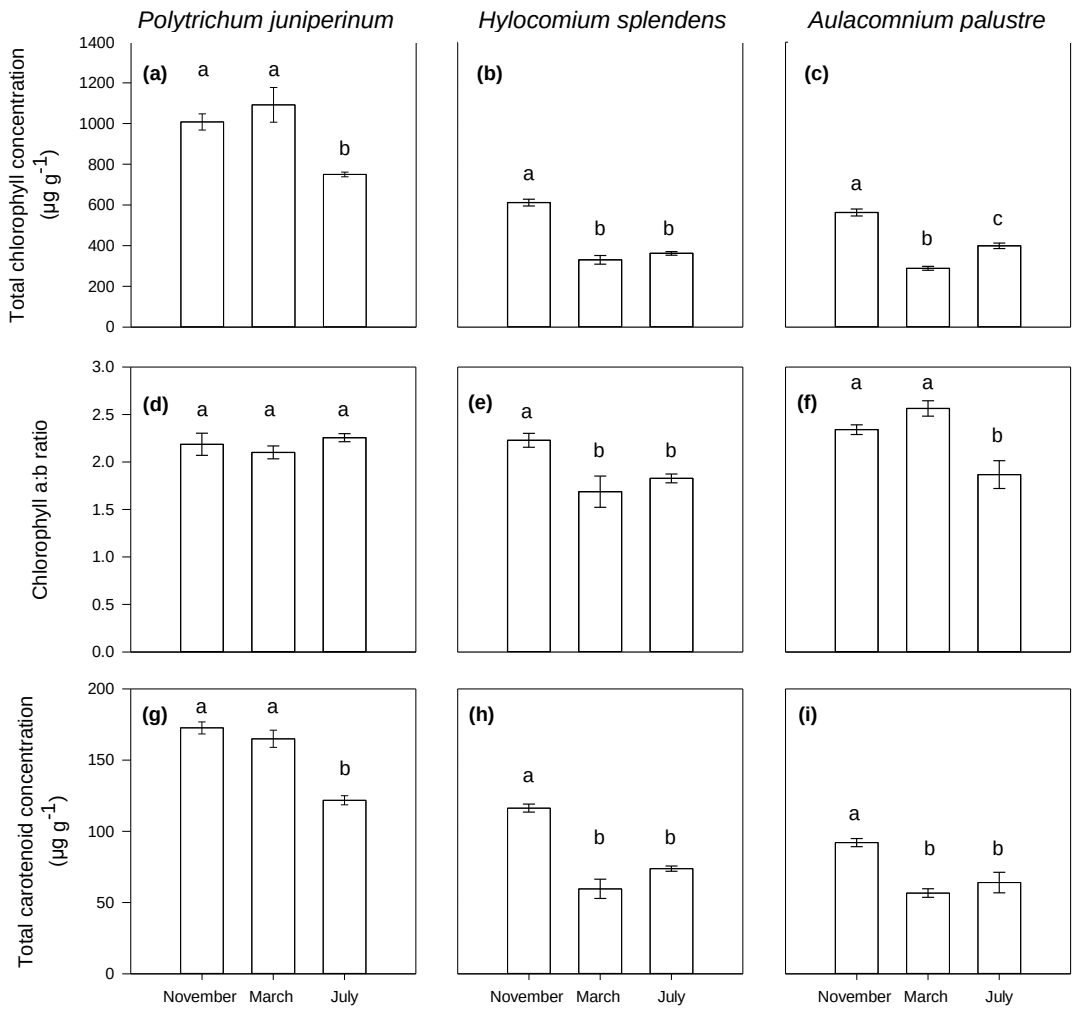

Figure 16: Mean values of total chlorophyll content (a,b,c), chlorophyll a:b ratio (d,e,f) and total carotenoid content (g,h,i), calculated from the analysis of light response curves of *Polytrichum juniperinum*, *Hylocomium splendens*, and *Aulacomnium palustre* at a range of ambient temperatures. Error bars represent +/- 1SEM. Different letters denote statistically significant differences at $p<0.05$.

Experiment 5: Spring temperature variation of the moss carpet

The average temperature of the moss carpet increased over the course of the monitoring period (Figure 17). The lowest temperature recorded during this time was -9.86 °C on the 24th of March and the highest temperature was 33.3 °C on the 18th of June. There were five days during the monitoring period when the temperature of the moss carpet did not exceed 0 °C, it is likely that significant snow cover was present during this time. The difference between the daily maximum and minimum temperature increased as the spring progressed. The largest daily min-max temperature difference was 30.5 °C, recorded on the 18th of April. The minimum temperature recorded on this day was -4.2 °C and the maximum was 26.3 °C. During the monitoring period there were 10 days when the min-max temperature difference exceeded 20 °C.

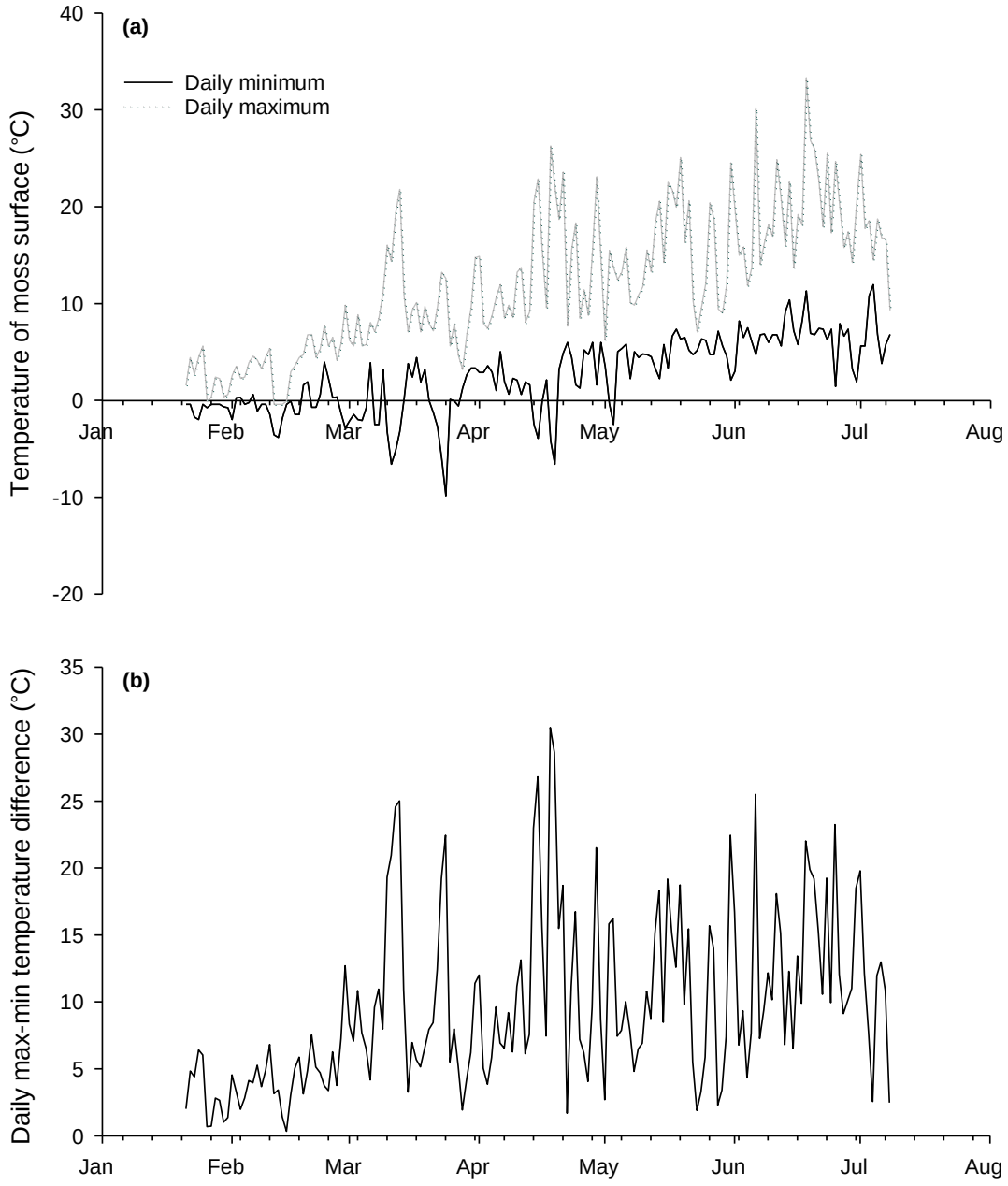

Figure 17*:* Daily temperature records measured at the moss surface at the UK field site between January and July 2014, (a) Moss surface temperature and (b) Daily temperature amplitude.

Experiment 6: Field measurements of whole-plant community-level gas exchange in *Hylocomium splendens*, *Aulacomnium turgidum* and *Tomentypnum nitens*

The aim of this experiment was to accurately measure gas exchange from mosses growing *in situ*. These results provide quantitative data on the carbon exchange capacity of these species under natural conditions, as well as valuable comparison to the data obtained in the laboratory.

Although the weather conditions over the two days when the 'warm' condition data were collected were very similar (12-13°C sun/cloud), the lack of temperature control inside the chamber meant that there were differences in both the chamber air temperature and the moss surface temperature between the different species studied (Table 4). There was also a high degree of variation in the relative amount of water that was lost from the different species between the 'wet' and 'dry' measurements, from an average of 28% loss in *A. turgidum* to a 64% loss in *H. splendens* and a 71% loss in *T. nitens*, due to the different physical structure and water-retention capabilities of these species. These factors, combined with the use of only three repeat samples in each condition (due to time restrictions) may explain the high variation and consequently few significant differences between conditions found in the analysis (Figure 18).

There was a significantly higher maximum rate of net photosynthesis in the warm-dry samples compared to the cool-wet samples of *H. splendens* ($p=0.007$). The light saturation point was also significantly higher in the warm-wet condition compared to the cool-wet condition in *H. splendens* ($p=0.018$). In *T. nitens* there was a lower rate of dark respiration in the warm-dry samples compared to the warm-wet samples ($p=0.041$), but no differences in the other factors between these conditions. There was also no significant difference between the warm-wet and warm-dry samples of *A. turgidum* in any of the parameters measured.

Table 4: Recorded state of the moss turves during field measurements.

Species	Condition	Average relative water content (%)	Moss surface temperature (°C)	Chamber air temperature (°C)
H. splendens	Warm-wet	375.51	14	15.0
	Warm-dry	134.79	12	17.5
	Cool-wet	480.28	6	8.5
A. turgidum	Warm-wet	282.75	17	14.5
	Warm-dry	202.89	15	15.5
T. nitens	Warm-wet	312.10	17	14.5
	Warm-dry	90.12	14	16.5

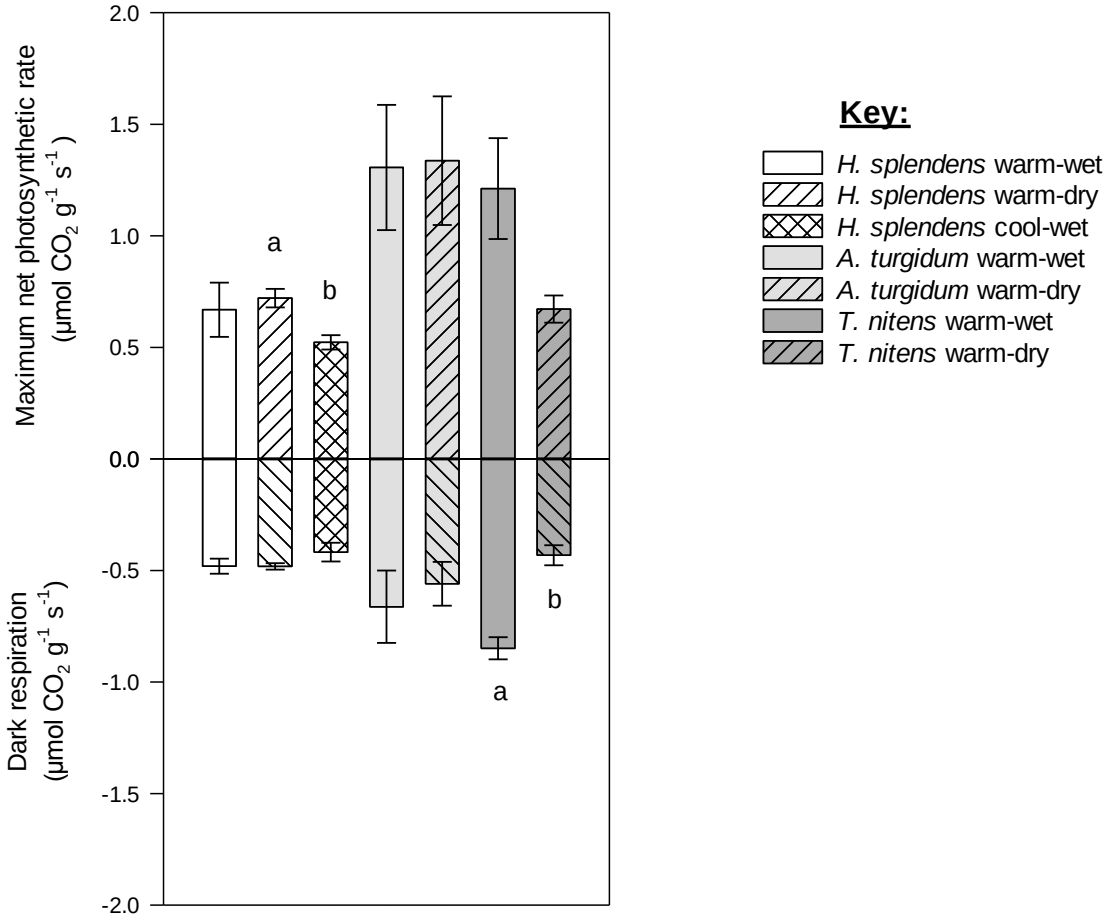

Figure 18: Mean values of maximum net photosynthesis and dark respiration calculated from analysis of light response curves of *Hylocomium splendens*, *Aulacomnium turgidum*, and *Tomentypnum nitens* under a range of environmental conditions. Error bars represent +/- 1SEM. Different letters denote statistically significant differences at the $p<0.05$ level.

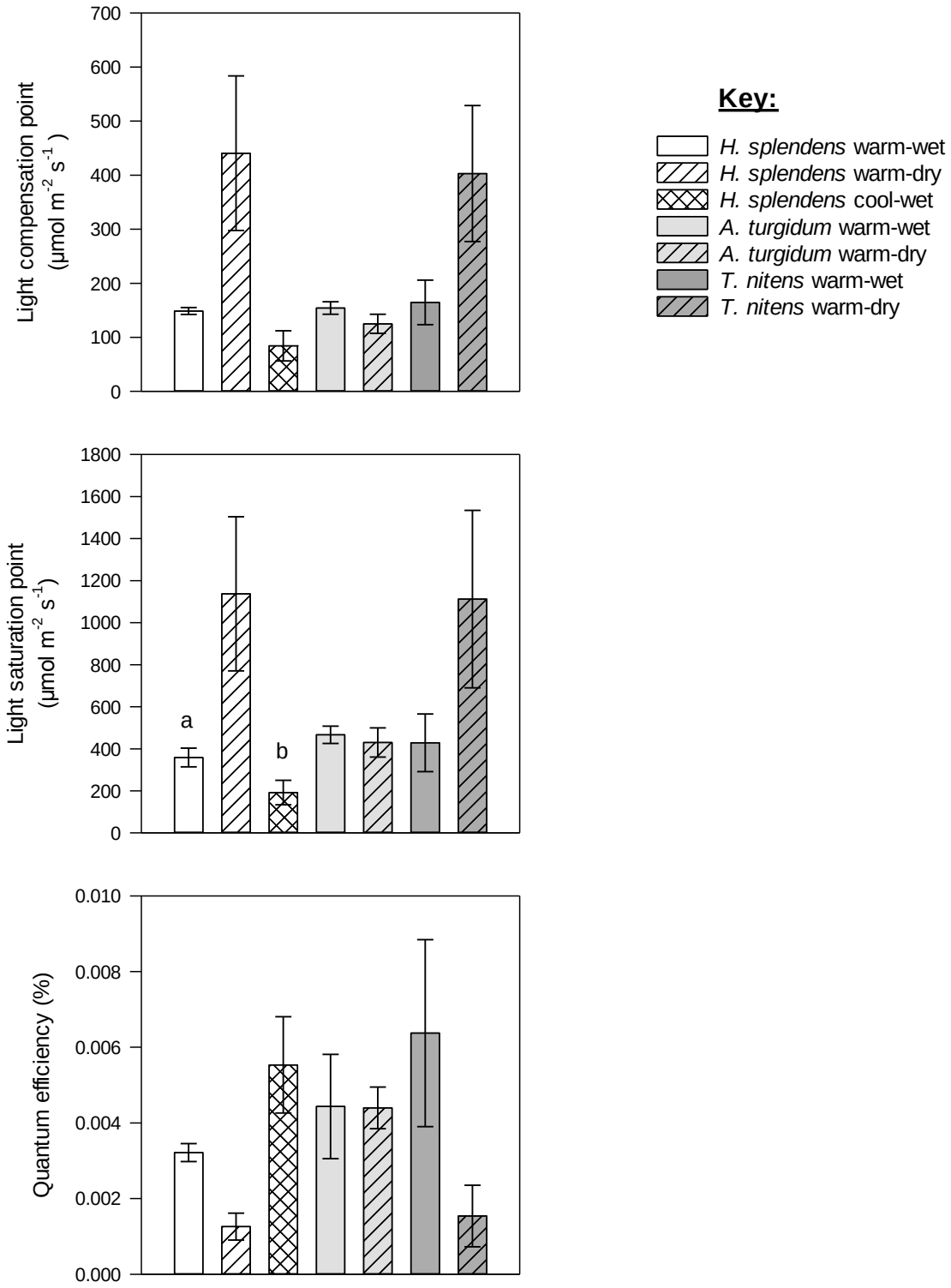

Figure 19: Mean values of the light compensation point, light saturation point, and quantum efficiency calculated from analysis of light response curves of *Hylocomium splendens*, *Aulacomnium turgidum*, and *Tomentypnum nitens* under a range of environmental conditions. Error bars represent +/- 1SEM. Different letters denote statistically significant differences at the $p<0.05$ level.

Chapter 4: Discussion

The aim of this study was to investigate the responses of a range of dominant boreal bryophyte species to changes in ambient temperature and water availability. This work is important if we are to understand the potential impacts of future climate scenarios on the bryophyte-dominated communities which are likely to be some of those most severely affected by climate change.

The effect of changing temperature on the photosynthetic activity of *Polytrichum juniperinum, Hylocomium splendens,* and *Aulacomnium palustre*.

This study has shown that it is possible to accurately measure significant positive rates of net photosynthesis in mosses at 1 °C using the LI-6400 bryophyte chamber. The relatively high rates of gas exchange measured at these low temperatures (up to 72% of the maximum rate in *Aulacomnium palustre*) support the existing evidence that many temperate and sub-arctic bryophyte species are adapted to allow significant growth at low temperatures (Zotz and Rottenberger, 2001; Bates et al., 2005). The next step in this area of study is to measure gas exchange at temperatures <0 °C, and consequently to find the minimum temperature at which significant photosynthesis is possible in these species. Several studies have shown that some bryophyte species are capable of measurable rates of photosynthesis down to -5 °C (Ino, 1990; Zotz and Rottenberger, 2001), however below this temperature metabolic activity appears to cease. A significant challenge in achieving this goal is likely to be the problem of increasingly high relative humidity inside the bryophyte chamber causing errors in CO_2 flux measurement. This was a noticeable problem when taking measurements at 1 °C, where the LI-COR built-in humidity scrub system was already used to its maximum capacity. It is likely that significant changes to the experimental protocol would be necessary in order to accurately measure at sub-zero temperatures.

The results of this study support previous findings that many bryophytes have a low optimum temperature for photosynthesis of *ca.* 10 °C (Uchida et al., 2002; Ino, 1990). This is significantly lower than vascular plants, which typically have an optimum temperature of 15 °C or higher, even in arctic environments. The exception

to this was *H. splendens,* which was the only species in this study to show a significant increase in A_{max} between 10 °C and 22 °C. Research suggests that the optimum photosynthetic temperature of some moss species changes seasonally. For example the optimum temperature for net photosynthesis of a sample of *H. splendens* from Alaska increased from 10 °C in early June to 25 °C in August due to higher rates of respiration early in the season (Skre and Oechel, 1981). It is therefore possible that different results would have been obtained in this study using moss samples collected in the spring or summer. Future studies should also include replicate samples taken from multiple moss turfs to ensure that the full range of physiological variation from within each population is measured.

The moss specimens used in the laboratory experiments were stored at 5 °C for the duration of the study. It has been documented that bryophytes, like vascular plants, are able to acclimate to the ambient temperature, and that this process alters their physiological responses to further temperature change (Sveinbjornsson and Oechel, 1983). It is therefore possible that the rates of photosynthesis measured in this study would also have been different if the plants had been exposed to the experimental temperature conditions for several days before measurements were taken. Further study could use this method to investigate how significant the effect of acclimation is on photosynthesis in these species.

The laboratory studies were limited by the small size of the bryophyte chamber which prevented the study of whole *P. juniperinum* and *H. splendens* plants. Using only < 1 year old tissue is common practice in the study of bryophyte photosynthesis as it reduces the error associated with using plants of varying age and quality, as well as increasing the accuracy of measurements where overall carbon flux for the whole plant is very small (Dilks and Proctor, 1979). The disadvantage of this practice is the difficulty it produces in scaling the results up to a community or ecosystem level. Photosynthetic pigments including chlorophyll are known to degrade during senescence of moss shoots (Tobias and Niinemets, 2010), and consequently rates of photosynthesis are significantly higher in < 1 year old shoots compared to the plant as a whole (Zotz and Rottenberger, 2001). In this study the maximum photosynthetic rates recorded for *P. juniperinum* and *H. splendens* were 3-4 x higher than the maximum rate recorded for *A. palustre*. It is not known

however how much of this difference was due to species differences, and how much due to the methodology used.

The effect of changing water content on the photosynthetic activity of *Polytrichum juniperinum* and *Hylocomium splendens*

The results of this study support previous findings that water content is a factor of equal or greater importance to temperature in bryophyte metabolism. A linear relationship between water content and net photosynthesis below the optimal water content is consistent with previous research in this area (Kennedy, 1993). In this study there was a strong positive linear correlation between the water content and net photosynthesis up to the maximum measured, with no evidence of a plateau or decline beyond the optimal water content as expected. The maximum rate of net photosynthesis of *H. splendens* was recorded at a water content of 230%, lower than the optimal water content of 425% reported by Skre and Oechel, (1981). This suggests that these samples may not have been fully hydrated as previously assumed. The value of 180% recorded for *P. juniperinum* in this study compares to an optimal water content of 100% measured in *Polytrichum commune* (Skre and Oechel, 1981), and 350% in *P. alpestre* (Kennedy, 1993). These results are consistent with the observation that *P. juniperinum* is adapted to maximise photosynthetic capability in a drier habitat than *H. splendens.*

In comparison, *H. splendens* was found to have a lower moisture compensation point than *P. juniperinum*. These results are consistent with the findings of Skre and Oechel, (1981) who recorded a moisture compensation point of 20% for *H. splendens* and 25% for *P. commune*. These values are however both lower than the 46% and 71% respectively, predicted by the results of this study. These results show that the response of these plants to moisture can vary significantly between closely-related species and according to the specific characteristics (age, location, growth form, etc.) of the sample tested. For example, Skre and Oechel (1981) used 2 year old shoots measured at 15 °C, compared to 1 year old shoots measured at 22 °C in this study. These factors may account for some of the difference in the results. To date little research has investigated the effect of water content on photosynthesis at very low temperatures (<5 °C), this would be a promising area for future research.

The effect of freeze-thaw conditions on the photosynthetic activity of *Polytrichum juniperinum* and *Hylocomium splendens*.

Sudden freezing events, especially following periods of spring warming, have been shown by several studies to reduce bryophyte growth in the field over the long term (Bjerke et al., 2011; Bokhorst et al., 2011). The results of this study suggest that *H. splendens* is better adapted to cope with freezing stress in the short-to-medium term than *P. juniperinum*. This is perhaps a surprising finding, considering the previous results of this study which suggest that the photosynthetic system of *P. juniperinum* is better adapted to low temperatures than that of *H. splendens*. Previous studies have also shown that *H. splendens* can be severely damaged by freeze-thaw events in the field (Bjerke et al., 2011). The cause of this discrepancy is likely to be the different conditions present in the two experiments, for example Bjerke et al recorded temperatures of -18 °C in the field compared to a minimum of -10 °C in this study. Further research is needed to determine the conditions under which *H. splendens* is able to tolerate freeze-thaw events, and the conditions under which it is damaged beyond recovery.

While *H. splendens* suffered minimal damage from the experimental freezing event, *P. juniperinum* suffered significantly greater damage. The data from the unshielded surface temperature logger deployed at the field site over the late winter/spring period show that while prolonged periods of sub-zero temperatures were rare at this site, daily cycles of freezing and thawing, with as much as a 30 °C difference between the maximum and minimum temperature over a 24 h period, were common. Although the 2013-14 winter was an unusually warm one in the UK, climate models predict that mild winters like this will become increasingly common in the future (IPCC, 2007). Studies have shown that short cycles of freezing and thawing are even more damaging to mosses than a single thaw period (Kennedy, 1993). These results therefore indicate that *P. juniperinum* could be seriously harmed by an increase in natural freeze-thaw events as a result of climate change.

Several studies have shown that mosses with low water content suffer reduced damage as a result of freezing compared to fully hydrated mosses (Schlensog et al.,

2004; Kennedy, 1993). In this study, a difference between the two conditions was present only in *P. juniperinum*. While there was no clear difference between the response of hydrated and dehydrated *H. splendens*, the overall effect of freezing on this species was barely significant in the present study. It is possible that under more severe conditions, where damage was greater, a difference would emerge between the hydrated and dehydrated conditions. Alternatively, the ability of *H. splendens* to tolerate freezing while hydrated with relatively little loss of photosynthetic capacity could be adaptive to the environment from which these samples were collected. *H. splendens* grows in thick, ground-level mats in sheltered locations which retain water. It is therefore more likely than *P. juniperinum* to be hydrated during freezing events and may have evolved greater tolerance to these conditions. In contrast, the individual shoots of *H. splendens* dry rapidly in the clear, dry air conditions which typically accompany high atmospheric pressure-associated cold weather in the UK. Further study would be helpful to determine whether sub-arctic populations of *P. juniperinum*, which must tolerate freezing periods in conjunction with large quantities of water from snow-melt, are better adapted to tolerate freezing while hydrated.

Seasonal variation in the chlorophyll content of *Polytrichum juniperinum*, *Hylocomium splendens*, and *Aulacomnium palustre*

The significant drop in chlorophyll content in *H. splendens* and *A. palustre* between November and March suggests that these species are suffering damage over the winter months, due, at least in part, to freezing stress (Skre and Oechel, 1981; Burke et al., 1976). A clear colour difference can be seen between moss collected in November and kept in the laboratory at 5 °C over the winter months, and moss collected from the field in March (Figure 20). Unfortunately chlorophyll measurements were not taken from the lab specimens, so a quantitative comparison is not possible here. This evidence suggests senescence in these species is partly triggered environmentally rather than occurring as an innate process. Measuring the spring chlorophyll content of these species in locations with very mild winters would help to determine if this is true *in vivo*.

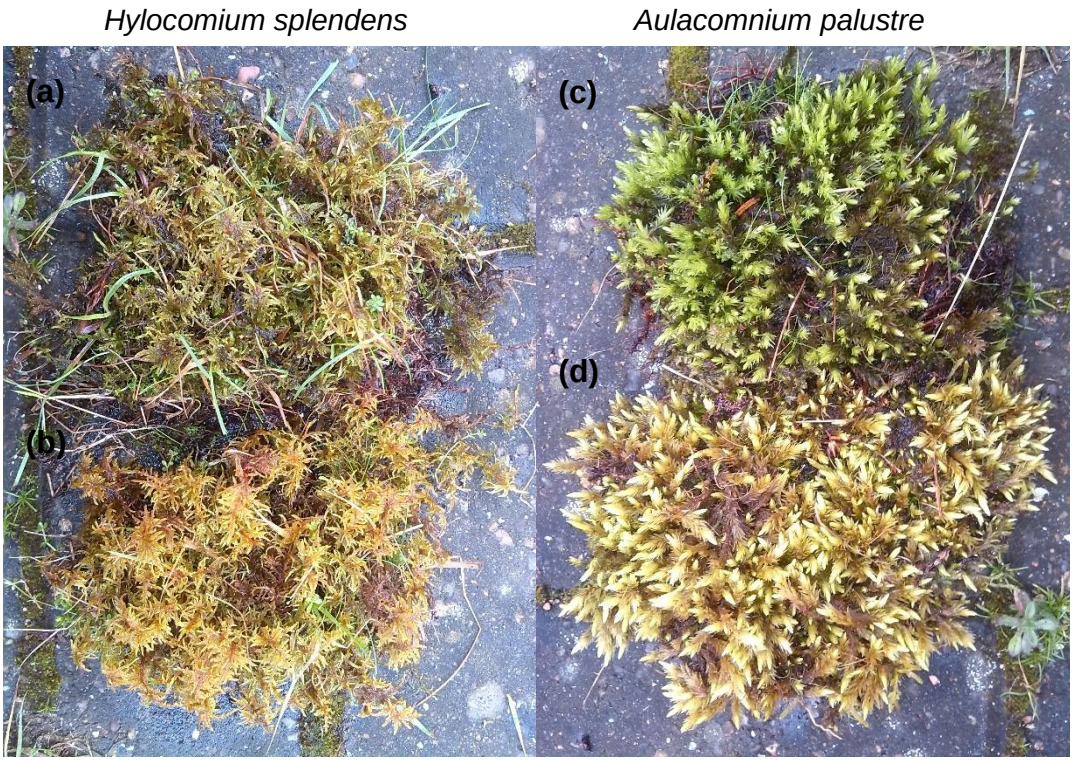

Figure 20: *Hylocomium splendens* and *Aulacomnium palustre* collected from the field in November (a, c), and March (b, d).

Unlike *H. splendens* and *A. palustre*, there was no reduction in the chlorophyll content of *P. juniperinum* over the winter months. If the reduction in chlorophyll content in *H. splendens* was due to freezing damage, then it is surprising that the same response was not seen in *P. juniperinum*, a species which was shown to be more sensitive to freeze-thaw events than *H. splendens* in this study. These results do however agree with the findings of Skre and Oechel (1981), who found that while the photosynthetic capacity of *H. splendens* fell over the winter, the overwintering leaves of *Polytrichum commune* maintained high photosynthetic capacity. Further research is needed into the physiological and molecular mechanisms associated with cold- and drought-tolerance in these species.

Chlorophyll content is known to be one of the major factors in determining the photosynthetic capacity of all plants, including bryophytes (Marschall and Proctor, 2004). The natural variation in seasonal chlorophyll content in *H. splendens* and *A. palustre* therefore highlights an important limitation of this study. All laboratory-based measurements of photosynthesis were conducted on material collected in November and early December. This means that the rates measured in these experiments cannot be extrapolated to other times of the year, when net

photosynthesis may be significantly lower than these experiments suggest. Further data should be collected at different times of the year to provide a more accurate picture of how these factors affect photosynthesis. In several species, the evidence suggests that autumn is often the most productive season (Zotz and Rottenberger, 2001; Skre and Oechel, 1981). In the long term climate change could significantly increase the productivity of these species if winter freezing damage is reduced, increasing the potential for significant spring growth.

Field measurements of whole-plant community-level gas exchange in *Hylocomium splendens*, *Aulacomnium turgidum* and *Tomentypnum nitens*.

At first glance, analysis of the data collected in the field in Canada appears to suggest that the moss species studied show little response to changing environmental conditions *in vivo*. However, due to the reliance of the experimental method upon natural changes in weather conditions, it was difficult to achieve consistently different conditions for study. This, combined with the use of only three replicate samples due to time constraints, resulted in a high degree of variation in the results. If the study were repeated over a longer time period, under multiple, more strictly controlled, temperature and moisture conditions, it is proposed that clearer differences would become apparent.

The finding that *H. splendens* had a higher rate of photosynthesis in warm-dry conditions compared to cool-wet conditions suggests that this species may benefit from warming even if water supply is not optimal. No significant difference was found between samples with 134% and 376% water content at 15-17 °C, suggesting that at this temperature the photosynthetic rate was constrained by a factor other than water content. If the sample was allowed to dry further, it is predicted that the photosynthetic rate would eventually fall. The fall in dark respiration rate between *T. nitens* samples of 312% and 90% water content is consistent with previous data.

Comparison between results from the laboratory and field sections of this study is difficult due to the different methods of measurement used. One clear difference however is the high rates of dark respiration relative to net photosynthesis measured in the field study. This is likely to be primarily due to the use of whole

plants, including large quantities of old material with a low photosynthetic rate, in the field study (Marschall and Proctor, 2004; Zotz and Rottenberger, 2001). This was also seen in the laboratory experiments when comparing the results from *A. palustre* to *P. juniperinum* and *H. splendens*. Another factor is likely to be the time of year. The field data were collected in the early season (June), whereas the laboratory data were collected from samples taken from the field in November. As the seasonal chlorophyll analysis shows, some moss species suffer significant damage over the winter due to freezing stress. Skre and Oechel (1981), found that the net photosynthesis of *H. splendens* in northern Sweden was close to zero in June, due to the high rates of respiration necessary for repair and growth at this time of year. Net photosynthesis then increased as the season progressed. It is likely that a similar process occurred with the samples of *A. turgidum* and *T. nitens* measured in this study. This difference is also reflected in the higher light compensation points recorded in the field compared to the earlier laboratory experiments.

Although extrapolation of laboratory data to the field environment is complex, the advantages of direct environmental manipulation and control can clearly be seen in the higher quality of this data compared to that collected in the field. The field data was severely limited by both practical difficulties and the weather conditions at the time of study, unlike the precisely controlled environment inside the laboratory. One way that these two strategies might be combined in future studies would be to use a larger carbon flux measurement chamber in combination with complete turfs of moss inside a climate controlled chamber. This strategy would combine many of the advantages of the two methods, and allow the investigation of factors which may have caused laboratory measured results to differ from those collected in the field.

Conclusion

The results of this study support the hypothesis that at temperatures in excess of freezing climate warming will result in increased moss photosynthetic activity. It is suggested that a longer growing season will have a greater positive impact on the carbon exchange capacity of mosses than an increase in summer temperatures, which may instead reduce photosynthesis rates due to increased evaporation from the moss carpet. The results also support the hypothesis that cycles of freezing and

thawing during the winter period negatively impact the photosynthetic capabilities of mosses compared to a single period of sub-zero temperatures. These results suggest that climate change will have a significant impact on the productivity, and consequently the carbon balance, of bryophyte-dominated ecosystems. The overall net effect, whether positive or negative, remains difficult to predict, and will depend greatly on the individual characteristics of species and communities. To fully understand the consequences of climate change in these ecosystems, it is necessary to accurately measure long-term gas exchange from a variety of different bryophyte species under a range of potential future climate scenarios.

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