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Rapid photosynthetic recovery of a snow-covered feather moss and *Peltigera* lichen during sub-Arctic midwinter warming

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Background: Arctic lichens and mosses are covered by snow for more than half the year and are generally considered as being dormant for most of this period. However, enhanced frequency of winter warming events due to climate change can cause increased disturbance of their protective subnivean environment.
Aim: To further understand cryptogamic responses to midwinter warming we compared the ecophysiological performance of one lichen and one moss species during a simulated warming event.
Methods: We measured photosynthesis and dark respiration in samples of the moss *Hylocomium splendens* and the lichen *Peltigera aphthosa* removed from under snow, and on natural refreezing after the warming event, which was simulated by using infrared heaters suspended above the ground.
Results: The moss exposed to light at +5 °C immediately after removal from their subnivean environment and from warmed plots showed positive net gas exchange within 332 s; the lichen required 1238 s. Photosynthesis and nitrogen fixation rates were equal to that, or higher than, during the preceding growing season. Upon refreezing after the event, moss photosynthesis declined considerably.
Conclusions: The moss, and to a lesser extent the lichen, may contribute to subnivean midwinter ecosystem respiration, and both are opportunistic, and can take advantage of warmer winter phases for photosynthesis and growth. This ought to be taken into account in vegetation change projections of cryptogam-rich ecosystems.

Keywords: carbon flux; climate change; cryptogams; dormancy; gas exchange; nitrogen fixation; reactivation; snow melt; subnivean environment; winter warming

Introduction

Drying and freezing may induce anabiosis in lichens and bryophytes. While the effects of rehydration and desiccation of bryophytes and lichens have received much attention (e.g. Smith and Molesworth 1973; Lange et al. 2006; Proctor et al. 2007), their freezing-induced anabiosis and reactivation – and cryobiology in general – are far less understood. The most cryotolerant lichens have detectable photosynthetic activity down to −24 °C (Lange 1965), but for most lichens, activity ceases at milder subfreezing temperatures (Kappen 1993). For bryophytes, photosynthesis has been reported down to -8 °C (Kappen et al. 1989). Low winter temperatures have been considered to halt functioning of cryptogams during the winter period (Phoenix and Lee 2004; Schlensog et al. 2004). However, considerable wintertime respiration suggests that many Arctic, sub-Arctic and alpine ecosystems are not at all dormant during winter (Zimov et al. 1993; Brooks et al. 1997; Grogan et al. 2001; Grogan and Jonasson 2006; Nobrega and Grogan 2007). Midwinter temperatures in the interface between snowpack and soil can be close to 0 °C, despite severe freezing temperatures above the snowpack (Grogan and Jonasson 2006; Bokhorst et al. 2010a), enabling subnivean metabolic respiration, especially by microbial soil organisms (Mikan et al. 2002).

Climate change in the Arctic is not only projected to lead to increases in mean wintertime temperatures, but also increased frequency of extreme warming events, which can result in rapid snow melt and loss of the insulating snow layer (Putkonen and Roe 2003; Christensen et al. 2007; Bokhorst et al. 2009; Callaghan et al. 2010, 2011a, 2011b). Both simulated and natural sub-Arctic winter warming events have recently been shown to cause considerable damage to plants (Bokhorst et al. 2008, 2009, 2010b, 2011, 2012). The most likely cause of such damage is the initiation of premature spring-like development, which is interrupted by return to normal winter temperatures, exposing the vegetation to freezing temperatures in the absence of an insulating snow cover (Crawford 2008; Bokhorst et al. 2010b). Lichens and bryophytes are important components of many Arctic and sub-Arctic vegetation types. In a recent winter warming simulation experiment (Bokhorst et al. 2008, 2011), it has been shown that the dominant lichen and bryophyte species had contrasting sensitivities to winter warming events; the feather moss *Hylocomium splendens* (Hedw.) Schimp. experienced severely reduced

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photosynthesis and growth rates during the following growing seasons, whereas the lichen *Peltigera aphthosa* (L.) Willd. remained unaffected (Bjerke et al. 2011). It was shown that the severe freezing following the warming events damaged vulnerable bryophyte tissues whose development was stimulated during the warming events (Bjerke et al. 2011), a similar mechanism as that seen to result in considerable damage to the vascular plants in the same experiment (Bokhorst et al. 2010b, 2011). These differences between the moss and the lichen in response to extreme winter warming indicate contrasting vulnerability to winter frost damage. Whether this is due to differences in ecophysiological activity is, however, not known, but there are indications of different recovery time after winter anabiosis for mosses and lichens (Schlensog et al 2004). Continental Antarctic bryophytes need more time to recover from winter anabiosis than lichens (Schlensog et al. 2004).

This paper originates from a winter warming manipulation experiment that was undertaken in the Swedish sub-Arctic. While the previous studies from this experiment focused on vascular plant and summertime cryptogamic responses to winter warming, this study focuses on wintertime responses of the dominant moss and lichen in this ecosystem. To explore midwinter reactivation rates of *H. splendens* and *P. aphthosa* and their associated cyanobacteria we measured ecophysiological activity of specimens that became gradually exposed during snow melt from a simulated extreme winter warming event, and of specimens removed directly from their subnivean environment. To the best of our knowledge, midwinter carbon flux measurements of sub-Arctic feather mosses and lichens and the nitrogen fixation activity of their associated cyanobacteria have not been reported previously. The results presented here therefore provide novel insight into the midwinter ecology of these cryptogams and their reactivation rates to winter warming events. Assuming that sub-Arctic and continental Antarctic bryophytes and lichens respond similarly, we hypothesised that the lichen would be reactivated more rapidly than the bryophyte in our study. We also hypothesised that the specimens that were gradually exposed would reach higher photosynthetic rates than the specimens removed directly from their subnivean environment, as the former group had more time to adapt to light. Finally, we expected that refreezing following the warming event would negatively affect photosynthetic capacity of the moss, as its hardening mechanisms were reduced during the warming event.

Materials and methods

Study area and species

This study was carried out in a sub-Arctic heathland close to the Abisko Scientific Research Station in northern Sweden (68° 21′ N, 18° 49′ E). The sub-Arctic heathland is dominated by evergreen dwarf shrubs (Bokhorst et al. 2008), but the most abundant lichen, *Peltigera aphthosa*, and bryophyte, the feather moss *Hylocomium splendens*, also have a high ground cover (Bjerke et al. 2011). In the study area, these two cryptogams are most abundant in mesic heath vegetation that under normal winter conditions are covered by snow for about 8 months (ca. October–May).

The warming treatment

Three discrete winter warming events were simulated, at the beginning of March (period of maximum snow depth in this region (Kohler et al. 2006)) in 2007, 2008 and 2009 by using infrared heating lamps to thaw the snow (for details see Bokhorst et al. 2008, 2009). The experiment consisted of 18 plots of 2.1 m × 1.0 m; six control plots and six of each of two warming treatments: canopy warming and canopy with soil warming. In the two warming treatments, four infrared heating lamps (Kalglo Electronics Co., Bethlehem, PA, USA) were suspended (70 cm apart) in parallel from wooden frames. The canopy with soil warming plots were further warmed by soil heating cables at 5 cm soil depth and running parallel at 20 cm distance from each other. Soil warming cables were switched on 2 days after the lamps to simulate the delay in soil thaw during a real event. Control plots received no warming treatment and remained insulated under the natural winter snow cover. Snow depth varied between 40 and 50 cm, and the soil surface temperature was around −3 °C (Bokhorst et al. 2010a). For this study, to avoid disturbing the control plots that served as control for the main experiment with the complete suite of species, we established new control plots for our measurements. Before snow fall in autumn 2008, sites with the two species close to the warming experiment were marked for use as control plots and revisited at the beginning of March in 2009. Temperatures were recorded by a data logger at 6-h intervals using thermistors placed in each plot at dwarf shrub canopy height (which was under snow prior to warming), at the soil surface and at 5 cm depth.

Each warming event lasted 7 days during which the lamps were kept at a constant distance of 50 cm from the snow surface, i.e. they were lowered as the snow depth decreased. This approach ensured a gradual snow thaw, taking 2–3 days to thaw the full depth of snow in each plot. As vegetation became exposed, lamps were kept at 50–70 cm above the soil surface to maintain canopy warming (lower lamp heights were needed during higher wind speeds and lower ambient temperatures). Temperatures from the thermistors were monitored to ensure warming was realistic and within the bounds of temperatures recorded for real events. The aim was to raise temperature to 5 °C (Bokhorst et al. 2008), and for most of the time temperature was close to 5 °C; temperature at canopy height fluctuated between 0.9 and 7.0 °C during the warming events. Thermocouple measurements of vegetation surface temperatures were also made to ensure that leaves did not overheat. Incident light (photosynthetic photon flux density; PPFD) was measured with quantum sensors (SKP215, Campbell Scientific, Shepshed, UK) placed at the ground (snow-covered in March) and at 1.5 m above ground (not covered by snow and with minimal shading from trees). The irradiance measurements at 1.5 m above

ground, representative also for the incident light to warmed plots after snow melt, reached daily maxima of between 166 and 290 μmol m^{-2} s^{-1} PPFD during the warming events in 2008 and 2009. Irradiance measurements on the ground under snow showed no light transmittance through the snowpack.

Sampling and ecophysiological measurements

During the third winter warming simulation event in March 2009 (i.e. of single events simulated in three consecutive years), metabolic activity in the lichen and bryophyte was measured by using a portable gas exchange and fluorescence system (GFS-3000, Heinz Walz GmbH, Effeltrich, Germany). Samples from the 12 warmed plots (both warming treatments) were measured 2–4 h after first emergence from under snow and exposure to warming treatment temperature while they still were moist from the melted snow. These samples were compared with those taken from below the snow in the control plots. The snow was carefully removed from the vegetation. Samples were collected one at a time, placed in dark bags and immediately brought to the GFS-3000 for measurements of dark respiration and photosynthesis. These samples henceforth are termed 'subnivean'. The temperature at the soil-snowpack interface at the time of sampling was around −3 °C (figure 1 in Bokhorst et al. 2010a). The time from sampling to the start of the gas exchange measurements was 3–4 min.

Samples were not artificially moistened; the melting snow and ice on their surfaces and within the thalli, and the relative humidity (RH) in the air were the only water sources for the subnivean samples, while the samples from the warming treatments were moist from the snow thawed by the heating lamps. The objective with not adding extra moisture was to test activity under natural thawing conditions. Samples were dried completely and weighed after measurements. Weights of naturally moist and dried samples showed that water content was within the range suitable for optimal photosynthetic rates (140–220% of dry weight). Only first-year and second-year segments of the feather moss were used. Each sample consisted of ca. five shoots. Lichen samples consisted of one ellipsoid lobe without apothecia, ca. 2.5 cm wide and 4 cm long.

While subnivean samples were naturally dark-adapted, warmed plot samples exposed to light were dark pretreated for 1 h before sampling. The analytical run consisted of a short period of instrument calibration in darkness (1 min), followed by measurements of dark respiration (DR) and maximal quantum efficiency of photosystem II (PSII), i.e. F_v/F_m (Maxwell and Johnson 2000), before the light was switched on. A saturating but not photoinhibiting (cf. Lange et al. 1996) PPFD of 400 μmol m^{-2} s^{-1} was used during measurements of net photosynthesis (NP). CO_2 concentration was set to 380 ppm, cuvette humidity to 7000 ppm H_2O, and temperature to 5 °C. This temperature was selected because it approximated the average canopy air temperature in the warming treatments after full snow melt (see figure 1 in Bokhorst et al. 2010b). During the light treatment, the quantum yield of PSII, Φ_{PSII} (Genty et al. 1989; Maxwell and Johnson 2000) and fluorescence quenching parameters were measured continuously (quenching data not reported here). Carbon assimilation curves flattened out after 5–45 min of light treatment (not to horizontal which would have needed more time for most samples, but until the steep, almost exponential rise in assimilation was passed). All samples were measured for at least 30 min in light. Assimilation rates were used to quantify the time taken from light exposure until positive net photosynthetic rates were reached, and to derive maximum NP rates (within the time limits and environmental conditions given; i.e. longer light treatments and/or higher temperatures would probably have rendered higher NP). DR (with negative values) and NP were used to calculate gross photosynthesis (GP), where GP = NP – DR. Values for NP, DR and GP were expressed on a dry weight basis. Comparisons with NP rates from the preceding growing season (reported in Bjerke et al. 2011) were used to check the potential of winter gas exchange; rates close to or higher than during summer would indicate high potentials.

To test how 1 day of freezing after the warming event would affect the photosynthesis and respiration (i.e. 12–18 h) after warming was turned off and before the first snowfall, samples of *H. splendens* moistened by wind-blown snow were collected from the warming treatments and measured using the same procedure as for the other samples. It took a full day to obtain a full data set. These samples are referred to as 'refreezing'. Capacity constraints on the GFS-3000 meant that only the moss could be analysed the first day after turning off the heat. The second day the warmed plots had been completely re-covered by newly fallen and wind-blown snow. Our principle was not to manipulate snow cover after the warming event. Hence, we could not dig for more samples, and the lichen was therefore not analysed after refreezing.

The leaf photosynthesis system used is supplied with a temperature sensor for measuring leaf temperature, but when using the cuvette specially designed for loose samples of cryptogams, this sensor is not in direct contact with the cryptogam. Thus, we cannot report exact thallus surface temperatures from the ecophysiological measurements.

Nitrogen fixation rates of cyanobacteria associated with *H. splendens* and *P. aphthosa* were measured during the second winter warming event in March 2008. Samples for nitrogen fixation measurements were randomly selected and carefully removed from the plots. They consisted of whole, cleaned thalli or tufts of ca. 25 cm^2 which were measured using the acetylene reduction assay (Stewart et al. 1967). No measurements on subnivean samples were taken. Samples were wetted and kept moist overnight. They were placed in air-tight chambers outdoors and incubated with 10% (v:v) acetylene for ca. 2 h (exact incubation time noted for every sample). Mean chamber temperatures (1–3 °C higher than ambient) and PPFD during incubation were 6.8 °C and 207 μmol m^{-2} s^{-1}. Gas samples were measured according to Zielke et al. (2002). Nitrogen fixation activity

during the event was compared with growing season fixation rates from the same plots reported in the electronic supplement of Bjerke et al. (2011).

Data analyses

Relationships between time of exposure to light and carbon assimilation rates were curve-fitted by using the sigmoidal Morgan–Mercer–Flodin model which, for all relationships, provided better fits than other models, both sigmoidal and non-sigmoidal. Differences between the two warming treatments were first tested with a series of Student's *t*-tests. As there were no significant differences between the two treatments for any of the measured parameters (lowest *P*-value was 0.11; most *P*-values were above 0.50), the two types of warming treatment data could be pooled (canopy only, and canopy plus soil warming), here called 'warming'. The pooled warming data were compared against subnivean samples and, for *H. splendens*, also against refrozen samples. Separate repeated-measures ANOVAs of warming vs. refreezing data rendered the same significance effects as when refreezing was considered a separate treatment in a one-way ANOVA. Thus, for being able to combine subnivean, warming and refreezing data in the same significance test, the results presented are based on one-way ANOVA with refreezing as a separate treatment. Post-hoc multiple comparisons of these data were analysed by using the Tukey–Kramer HSD test. A two-way ANOVA was used to test for significant species × treatment interactions on response rates. Student's *t*-tests were used to compare subnivean and warming data of *P. aphthosa*, and a paired Student's *t*-test was used to compare warming treatment NP from March 2009 and July 2008.

Data sets were tested for heterogeneity using Levene's test. In cases where this test was significant, suggesting lack of homogeneity, the data were also analysed by using non-parametric tests (the Kruskal–Wallis and Mann–Whitney *U* tests). Changing from parametric to non-parametric tests did not affect significance in any of the cases, i.e. in cases where *P*-values were below 0.05 using ANOVA, significance levels were below 0.05 also with the non-parametric tests, and vice versa. All tests were carried out by using the PASW Statistics 18 package (SPSS Inc., Chicago, IL, USA), except for the curve fitting, which was made in Microsoft Excel by using the add-on XLfit ver. 5.3.1.3 (ID Business Solutions Ltd., Guildford, UK).

Results

Response times to light exposure

Positive photosynthetic rates of *Hylocomium splendens* were reached within an average of 332 s. The three sample types of *H. splendens*, i.e. samples from the subnivean environment, the warming plots and upon refreezing 1 day after warming, showed similar time responses to light exposure (Figure 1; $F_{2,23} = 0.06$, $P = 0.94$). *Peltigera aphthosa* showed a large variation in response times, with samples from the subnivean environment being on average nearly

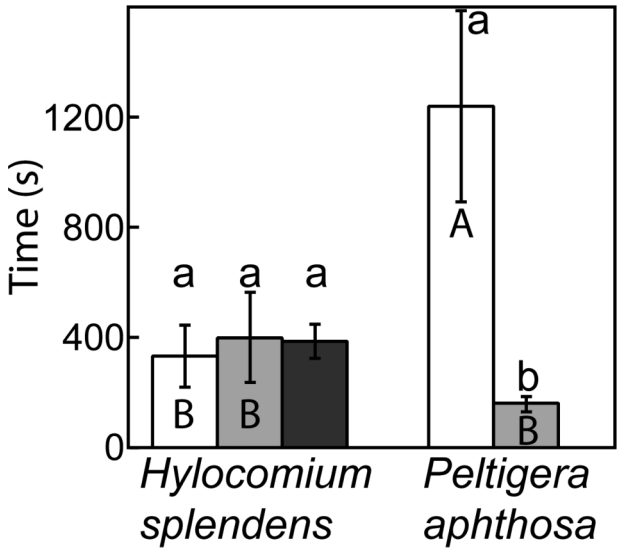

Figure 1. Time from start of light exposure (400 μmol m^{-2} s^{-1} PPFD) until positive photosynthetic rates were reached for *Hylocomium splendens* and *Peltigera aphthosa* at 5 °C during a winter warming event in March 2009 (light grey bars), upon refreezing 1 day after warming (only *H. splendens*; dark grey bar) and of samples dug out from under snow (subnivean control; unfilled bars). Error bars are ± SE. Lower-case letters above the columns indicate significant differences among means from the same species, whereas upper-case letters in the columns indicate significant interspecific differences among means from the same sample type.

eight times slower than samples from the warming treatment (Figure 1; lack of homogeneity; Mann–Whitney *U* test, $P = 0.005$). Subnivean samples of *P. aphthosa* needed on average 1238 s to reach positive photosynthetic rates. Samples of the lichen and the moss from the warming treatment had similar response times to light exposure, while subnivean samples of the lichen had significantly longer response times than subnivean samples of the moss (Figure 1, upper-case letters at the columns, interaction species × treatment: $F_{2,24} = 12.04$, $P = 0.002$).

Typical response curves of photosynthetic rates as a function of time since first light (Figure 2) show that the fittest samples of *H. splendens* reached positive rates after 60 s of light exposure (Figure 2(a); canopy warming example). After refreezing, a few samples tended to respond more slowly to the light treatment (example with open squares in Figure 2(a)); albeit without having an effect on mean response times for this group (Figure 1). The distinctive differences in response times between lichen samples from the warming treatment and from the subnivean environment is exemplified by three samples in Figure 2(b). The samples with the fastest response reached maximal NP within ca. 600 s, as seen from the curve flattening of the canopy and soil warming example in Figure 2(b).

Ecophysiological performance

Overall, ecophysiological performance of *H. splendens* was identical in the subnivean and winter warming samples,

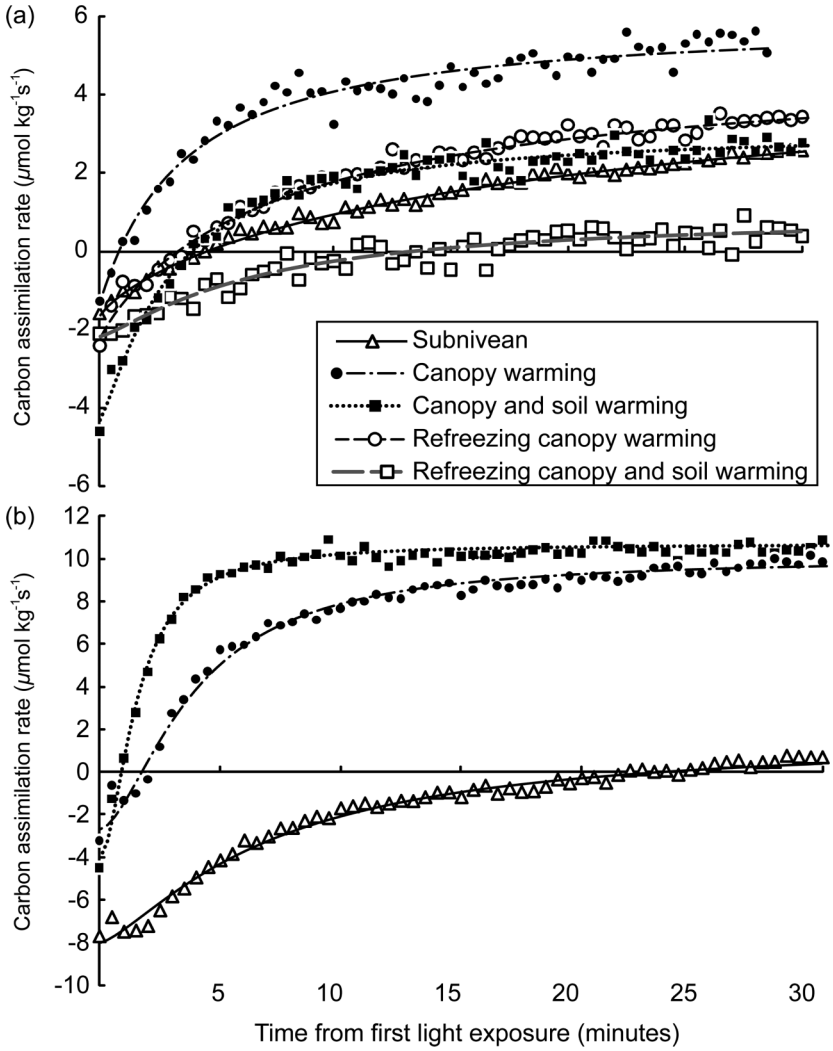

Figure 2. Examples of typical response curves for individual thalli of *Hylocomium splendens* (a) and *Peltigera aphthosa* (b) during the first 30 min of exposure to light (400 μmol m^{-2} s^{-1} PPFD). Only *H. splendens* was measured after refreezing. Correlation coefficients (R^2) are between 0.96 and 0.98 for the fitted sigmoidal regression curves, except for the refreezing canopy and soil warming curve ($R^2 = 0.90$).

but refreezing samples differed (Figure 3, left panels). In *H. splendens* DR in the subnivean, warming, and refreezing samples was variable and there were no differences among the three treatments (Figure 3(a); $F_{2,23} = 0.14$, $P = 0.87$). Upon refreezing large declines were found in NP and Φ_{PSII}. NP in refrozen samples was 59% lower (Figure 3(b); $F_{2,23} = 6.01$, $P = 0.009$) and Φ_{PSII} was 2.5 times higher (Figure 3(d); $F_{2,23} = 8.99$, $P = 0.002$) compared with subnivean samples. Mean F_v/F_m was 14.5% lower upon refreezing than during the warming event (Figure 3(c); lack of homogeneity; Kruskal–Wallis, $P = 0.069$). Mean NP of *H. splendens* during the winter warming event in 2009 did not differ from growing season NP (paired $t_7 = 1.05$, $P = 0.33$).

DR of *Peltigera aphthosa* was 1.7 times higher in subnivean samples compared with the winter warming treatment (Figure 3(a); $F_{1,12} = 10.09$, $P = 0.009$), and chlorophyll fluorescence was 35% lower compared with the winter warming treatment (Figure 3(c); lack of homogeneity, Mann–Whitney U test, $P = 0.009$). Mean NP was 57% lower in the subnivean samples compared with the winter warming treatment, but due to high variability not significantly so (Figure 3(b); $F_{1,12} = 3.23$, $P = 0.1$), and the same applies to Φ_{PSII} which was 37% higher in the subnivean samples (Figure 3(d); $F_{1,12} = 3.78$, $P = 0.078$). NP of *P. aphthosa* during the winter warming event in 2009 was on average 4.3 times higher than during the preceding growing season (paired $t_6 = -3.78$, $P = 0.009$).

GP of the two species did not differ among the treatments (*H. splendens*: $F_{2,23} = 1.43$, $P = 0.26$, *P. aphthosa*: $F_{1,12} = 0.88$, $P = 0.37$; data not shown). Nitrogen fixation activity was high during the second winter warming event, with mean values of 1.26 and 2.23 mmol C_2H_4 h^{-1} g^{-1} for *H. splendens* and *P. aphthosa*, respectively (no differences between groups, data not shown), for both species being more than twice as high as the activity measured in July the preceding year (all treatments pooled; *H. splendens*: $t_{29} = -2.23$, $P = 0.034$; *P. aphthosa*: $t_{23} = -4.04$, $P = 0.001$).

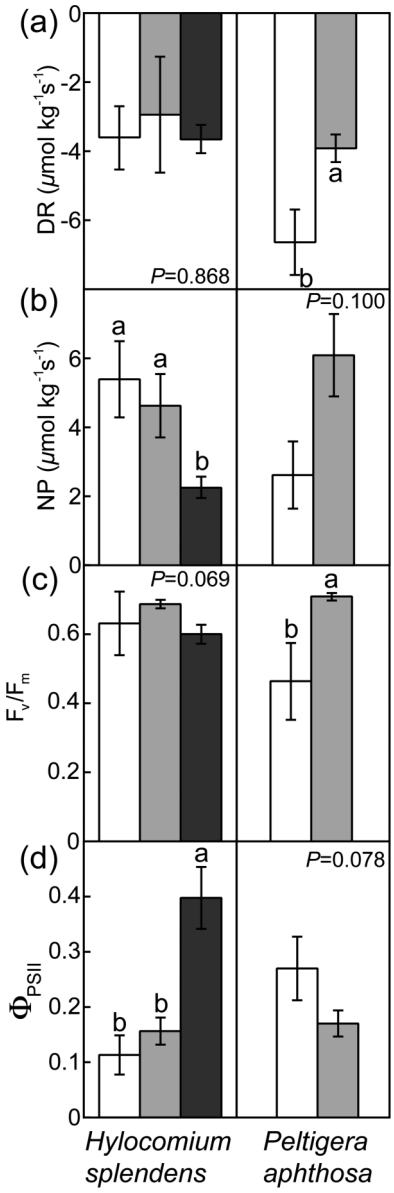

Figure 3. Ecophysiological performance of *Hylocomium splendens* (left) and *Peltigera aphthosa* (right) at 5 °C during the winter warming event in March 2009 (light grey bars), upon refreezing after warming (only *H. splendens*; dark grey bar) and of samples dug out from under snow (subnivean; unfilled bars). (a) DR; (b) NP; (c) F_v/F_m; (d) Φ_{PSII}. Error bars are ± SE. Different letters indicate significant differences ($P < 0.05$) between means. The exact *P*-levels are given for cases without significant differences.

Discussion

Our results suggest that on exposure to light and temperatures above freezing the moss responded nearly four times faster than the lichen to gain positive NP following a number of months of darkness under snow. This is in contrast to what we expected, as Schlensog et al. (2004) found that bryophytes were slower to recover than lichens in continental Antarctica. The physiological measurements suggest that sub-Arctic bryophytes and lichens can contribute significantly to winter ecosystem respiration and assimilation, as also recently suggested by Street et al. (2012) based on primary productivity analyses during late winter and spring of the two bryophytes *Polytrichum piliferum* Hedw. and *Sphagnum fuscum* (Schimp.) H. Klinggr.

The average response time of 332 s by *H. splendens* was particularly rapid, but the response by *Peltigera* at 1238 s was also rapid in comparison with the Antarctic bryophyte *Bryum subrotundifolium* A. Jaeger which needed 16 h from first reactivation after overwintering under a 30 cm-deep snowpack before positive NP was gained (Schlensog et al. 2004). While the temperature of the subnivean Antarctic environment at -15 °C (Schlensog et al. 2004) was too low for any significant cryptogamic metabolic activity (Kappen 1993), the sub-Arctic subnivean environment in this study had a temperature of −3 °C, which is above the lower limit for metabolic activity. This difference in the degree of dormancy is the most likely cause for the contrasting response times between the Antarctic and the sub-Arctic sites.

The lack of difference in NP, DR, F_v/F_m and Φ_{PSII} between the subnivean control and the warming treatments demonstrated that *H. splendens* was not at all dormant in its subnivean environment. As the subnivean microclimate in the sub-Arctic is suitable for high water potentials (Zimov et al. 1993; Mikan et al. 2002; Grogan and Jonasson 2006; Nobrega and Grogan 2007), this suggests that subnivean bryophytes may significantly contribute to wintertime CO_2 respiration rates. This contrasts with the situation in continental Antarctica where persistently low winter temperatures make wintertime water potentials very low, even at high subnivean RH, leading to extensive desiccation at the cellular level (Schroeter et al. 1994; Schroeter and Scheidegger 1995).

Differences between H. splendens *and* P. aphthosa

High DR rates of subnivean *P. aphthosa* (Figure 3(a)) indicate that the lichen also has the potential of subnivean respiration when temperatures are close to 0 °C and may therefore contribute to wintertime ecosystem respiration, depending on the temperature course. Several lichens show detectable DR under mild subfreezing conditions (e.g. Gannutz 1970; Lange and Green 2005). Mild subnivean conditions are in fact suggested as a primary reason why terricolous, fruticose lichens are very sparse in oceanic areas of the Arctic and sub-Arctic, because such dark and mild conditions over several months may cause severe respiratory loss that can ultimately kill the lichen (Bjerke 2011). These lichens are often more abundant in continental areas with lower subnivean temperatures, where they make up an important part of the winter forage for reindeer (e.g. Tømmervik et al. 2012).

Lichens tend to rapidly release a burst of non-metabolic CO_2 the first 15 min during a temperature increase (Sundberg et al. 1999). The lichens from the subnivean environment experienced a rapid temperature increase of 8 °C (from −3 °C to +5 °C) while being transported from the field to the gas exchange chamber, whereas the samples from the warmed plots had been at 5 °C for some hours

prior to gas exchange measurements. The temperature increase that the subnivean samples were exposed to certainly led to a burst of CO_2 release, and this explains why DR of *P. aphthosa* was higher in the subnivean samples than in the samples from the warmed plots (Figure 3(a)), which had its burst release of CO_2 while being heated up in the plots a few hours before gas exchange measurements.

We suspect that the longer response times of the lichen compared with the moss were due to their large differences in surface area-to-volume ratios. Thick, broad-lobed foliose lichens such as *P. aphthosa* have much lower ratios than feather mosses, and this leads to higher water retention which, in turn, slows down the thawing rate. Thus, the moss probably reached positive thallus temperatures much faster than the lichen when they were moved from their subnivean environment at around −3 °C to the cuvette temperature at +5 °C. Street et al. (2012) also used differences in water retention capacity to explain why *Sphagnum fuscum* has lower photosynthetic rates than *Polytrichum piliferum* in late winter, as large amounts of frozen water within capillary spaces of *S. fuscum* melt slowly and restrict CO_2 diffusion. The longer response time and the reduced subnivean F_v/F_m (Figure 3(c)) of *Peltigera aphthosa* as compared to *H. splendens* indicate that the high water retention of the lichen slowed down the reactivation rate after light exposure. Subnivean samples of an Antarctic liverwort have also been reported to have had much lower chlorophyll fluorescence than adjacent samples that were free of snow (Snell et al. 2007). Nevertheless, the short time required to reach positive NP shows that *P. aphthosa* can take advantage of winter thawing events for photosynthesis and growth, and lichens with higher surface area-to-volume ratios, e.g. fruticose reindeer lichens (*Cladonia* spp.), may thaw more rapidly and be more similar to *H. splendens* than to *P. aphthosa* in terms of response time.

Comparison with growing season activity

NP and nitrogen fixation rates of *H. splendens* and *P. aphthosa* during the growing season in the study area are variable (Bjerke et al. 2011). NP rates of *P. aphthosa* and N fixation rates of both species during the winter warming event were 2–4.3 times higher than the range of rates during the preceding growing season, suggesting that the winter warming events rendered optimal temperature and humidity conditions for ecophysiological activity. In fact, it has been suggested that many sub-Arctic cryptogams have the highest photosynthetic activity during late winter, spring and autumn, because thalli stay moist for longer periods of time during these seasons due to water from snowmelt, higher precipitation rates and slower drying rates than during summer (e.g. Sonesson 1989, 2001; Rikkinen 1995; Moore et al. 2002; Bjerke et al. 2005). This may be especially true for continental parts of the circumpolar region which can be very dry and warm in summer. For example, the maximum photosynthesis rates of the feather moss *Pleurozium schreberi* (Brid.) Mitt. from Finland were much higher in spring and autumn than in summer (Kallio and Saarnio 1986), whereas the epiphytic lichens *Melanohalea olivacea* (L.) O. Blanco et al. and *Parmeliopsis ambigua* (Wulfen) Nyl. from the Abisko area showed much higher growth rates in spring than in summer and autumn (Sonesson et al. 2011). Also, in warmer and wetter regions, for example the British Isles, the cold seasons are considered an important period for cryptogamic growth, due to continuously moist conditions (Bates et al. 2005). Our results indicate that *P. aphthosa* and cyanobacteria may also be more active in autumn and spring, rather than during summer, but to confirm this, year-round monitoring of carbon exchange would need to be carried out, as was done with the temperate lichen *Lecanora muralis* (Schreber) Rabenh., whose carbon assimilation was almost completely dependent on momentary hydration conditions (Lange 2003). NP in *Hylocomium splendens* was not different from NP during the preceding summer, and this contrasts to the results for NP in *P. aphthosa* and N fixation in the cyanobacteria. This may be due to the fact that the mosses in the warmed plots were damaged by the winter warming events of 2007 and 2008 (Bjerke et al. 2011), and therefore the subnivean moss samples required more time to reach maximum NP rates.

Refreezing

High ecophysiological activity and spring-like development generally lead to de-hardening (e.g. Rütten and Santarius 1992; Ögren 1996; Bokhorst et al. 2010b), and the lichen and moss therefore run a risk of damage by refreezing, a risk which is higher for mosses due to their freeze-susceptible organs (Clausen 1964; Hudson and Brustkern 1965; Kennedy 1993; Bjerke et al. 2011). Refreezing led to a reduction of the photosynthetic performance of *H. splendens*; NP was reduced by 52%, F_v/F_m was near-significantly reduced, and Φ_{PSII} was 1.5 times higher than during the event (Figure 3). Φ_{PSII} measures the proportion of light absorbed by chlorophyll associated with PSII that is used in photochemistry, and it often shows an inverse correlation with the efficiency of carbon fixation (Genty et al. 1989; Maxwell and Johnson 2000). Thus, the higher refreezing values of Φ_{PSII} indicate reduced efficiency, as also demonstrated by the reduced NP. Moreover, NP after refreezing was lower and Φ_{PSII} higher than the subnivean values, suggesting that refreezing imposed stress causing stronger reductions than seen after the down-regulation of activity during winter dormancy. Bjerke et al. (2011) hypothesised that the high sensitivity to extreme winter warming by *H. splendens* seen during the following growing seasons was because of initiation during the warming events of growth of young, freeze-susceptible shoot apices, which were damaged on refreezing after the warming event. The ecophysiological data presented here confirm that the moss was active during the warming events, and that freeze-induced stress immediately after the warming events caused severe reductions in ecophysiological performance. However, new growth during the warming event could

not be observed visually. Therefore, to clearly confirm that growth was initiated during extreme winter warming events, it would have been necessary to assay biochemical responses related to growth, as was made for vascular plants in the same warming simulation (Bokhorst et al. 2010b).

Our data show that the lichen *P. aphthosa* was highly active during the winter warming event but, presumably, as this lichen does not have any freeze-susceptible organs, it could withstand the sudden post-warming refreezing without being damaged (Bjerke et al. 2011). Nevertheless, it would be relevant to test if *P. aphthosa* also experiences a sudden reduction in photosynthetic performance upon refreezing.

Conclusion

The results presented here provide increased insight to the winter ecology of heath cryptogams in the sub-Arctic. Their moist and relatively mild subnivean environment prevents full dormancy, at least for parts of the winter season. Instead, they probably have some more or less continuous respiratory activity while staying ready to take advantage of solar radiation as soon as light transmittance through snow is above the light compensation point for photosynthetic activity, which for cryptogams is generally reached at 17–30 μmol m^{-2} s^{-1} PPFD (Kappen 1993; Lange et al. 1996; Sommerkorn 2000; Pannewitz et al. 2003; Street et al. 2012). Thus, their role in wintertime carbon fluxes may have been underestimated. Full snow melt and increases in temperature to a few degrees above freezing, as experienced during the winter warming events, are shown to render good conditions for ecophysiological activity, leading to NP and nitrogen fixation rates similar to or larger than typical rates observed during the growing season. Winter climate change with increasing frequency of extreme warming events may, therefore, have large consequences for summer growth of lichens and mosses. It may affect their competitive potential against vascular plants which are known to be highly sensitive to winter warming events (Bokhorst et al. 2008, 2009, 2010b, 2011, 2012; Crawford 2008; Callaghan et al. 2010, 2011a, 2011b). This suggests that winter processes may reduce the rate of increasing dominance of vascular plants over cryptogams resulting from summer processes which stimulate vascular plant growth (Cornelissen et al. 2001; Keuper et al. 2011). In fact, the balance between winter and summer processes is unknown and is a major topic for future research. Enhanced knowledge of the winter ecology of cryptogams is, in this context, crucial for the understanding of the full impacts of climate change in polar regions. We have here shown that sub-Arctic lichens and mosses are not as dormant in midwinter as previously assumed. This implies that increased opportunities for growth by cryptogams during the cold seasons, due to increased frequency of warming events, must be taken into account when modelling future vegetation composition changes in the sub-Arctic.

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