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1 **Cold acclimation in warmer extended autumns impairs freezing tolerance of freezing tolerance**
2 **of perennial ryegrass (*Lolium perenne* L.) and timothy (*Phleum pratense* L.)**
3

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5 **Sigridur Dalmannsdottir^{a,b}, Marit Jørgensen^{a,*}, Marcin Rapacz^c, Liv Østrem^d, Arild Larsen^e,**
6 **Rolf Rødven^{a,f} and Odd Arne Rognli^b**
7

8 ^aNorwegian Institute of Bioeconomy Research, NIBIO, Holt, 9016 Tromsø, Norway

9 ^bDepartment of Plant Sciences, Norwegian University of Life Sciences, NMBU, 1432 Ås, Norway

10 ^cUniversity of Agriculture in Krakow, Department of Plant Physiology, Podluzna 3, 30-239 Krakow,
11 Poland

12 ^dNorwegian Institute of Bioeconomy Research, NIBIO, Fureneset, 6967 Hellevik i Fjaler, Norway

13 ^eGraminor Ltd., Torggården, 8049 Bodø, Norway

14 ^fFaculty of Bioscience, fisheries and economy, UiT –The Arctic University of Norway, 9037 Tromsø,
15 Norway
16

17
18 *Corresponding author, e-mail: marit.jorgensen@nibio.no
19
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1 The effect of variable autumn temperatures in combination with decreasing irradiance and daylength
2 on photosynthesis, growth cessation and freezing tolerance was investigated in northern- and southern-
3 adapted populations of perennial ryegrass (*Lolium perenne* L.) and timothy (*Phleum pratense* L.)
4 intended for use in regions at northern high latitudes. Plants were subjected to three different
5 acclimation temperatures; 12, 6 and 9/3°C (day/night) for four weeks, followed by one week of cold
6 acclimation at 2°C under natural light conditions. This experimental setup was repeated at three
7 different periods during autumn with decreasing sums of irradiance and daylengths. Photoacclimation,
8 leaf elongation and freezing tolerance were studied. The results showed that plants cold acclimated
9 during the period with lowest irradiance and shortest day had lowest freezing tolerance, lowest
10 photosynthetic activity, longest leaves and least biomass production. Higher acclimation temperature
11 (12°C) resulted in lower freezing tolerance, lower photosynthetic activity, faster leaf elongation rate
12 and higher biomass compared to the other temperatures. Photochemical mechanisms were
13 predominant in photoacclimation. The northern-adapted populations had a better freezing tolerance
14 than the southern-adapted except when grown during the late autumn period and at the highest
15 temperature; then there were no differences between the populations. Our results indicate that the
16 projected climate change in the north may reduce freezing tolerance in grasses as acclimation will take
17 place at higher temperatures and shorter daylengths with lower irradiance.
18

19 **Key words:** Cold acclimation, chlorophyll fluorescence, freezing tolerance, leaf elongation,
20 geographically adapted populations, climate change

21 *Abbreviations* - F_m (F'_m), maximal chlorophyll fluorescence yield in the dark-adapted (light-adapted)
22 leaf; F_o (F'_o), minimum chlorophyll fluorescence yield in the dark-adapted (light-adapted) leaf; F_s ,
23 steady-state chlorophyll fluorescence yield in the light-adapted leaf; F_v , $F_m - F_o$; ϕ_{PSII} , current quantum
24 yield of PSII; q_p , coefficient of the photochemical quenching of chlorophyll fluorescence; NPQ, non-
25 photochemical quenching of chlorophyll fluorescence.

26

1 **Introduction**

2 Autumn and winter temperatures are predicted to increase considerably at higher northern latitudes the
3 coming decades (ICPP 2013). This in combination with the low irradiance and short day length at
4 these latitudes can intensify or give new problems with overwintering of perennial forage crops.
5 Rising winter temperatures and fluctuating weather conditions with unstable snow cover could
6 increase the winter stresses of plants (Bertrand and Castonguay 2003, Uleberg et al. 2014, Cooper
7 2014). The predicted changes in autumn climate may affect cold acclimation of perennial plants
8 negatively.

9 Cold acclimation of herbaceous species has been widely studied (reviewed by e.g. Thomashow 1999,
10 Cinnusamy et al. 2006, Sandve et al. 2011, Quellet and Charron 2013, Wingler 2015) and is a process
11 where climatically adaptive plants can increase their freezing tolerance in response to low non-
12 freezing temperatures. Freezing tolerance is a dynamic character affected by environmental factors
13 such as temperature and light (Gray et al. 1997) and is both seasonally (Yoshida et al. 1997, Palva et
14 al. 2002) and diurnally (Keily et al. 2013) controlled. A decrease in temperature during late
15 summer/early autumn triggers changes in the gene expression, resulting in increased freezing tolerance
16 of the plant (Cinnusamy et al. 2006). In the plant cell, the chloroplast may be the primary site for cold
17 sensors of ambient temperatures in addition to the plasma membrane (Miura and Furumoto 2013). As
18 reviewed by Hüner et al. (2014), the formation of an excitation pressure within photosystem II (PS II)
19 in photosynthetic active tissue as a response to decreasing temperatures is the sensor for cold regulated
20 mechanisms; not the low temperature per se. Excitation pressure develops as a response to over-
21 reduction of PS II since electron transfer through the electron transport chain is too slow (Hüner et al.
22 2013). This situation occurs either as a response to low temperature, which reduces the rate of carbon
23 assimilation and hence the need for photochemical energy; or as a response to high light conditions
24 (Ensminger et al. 2006). This redox sensing signalling through excitation pressure is both species and
25 cultivar dependent (Hüner et al. 2013). Adaptive genotypes can avoid photoinhibition and start a
26 process of photoacclimation either by increasing the rate of energy dissipation by non-photochemical
27 quenching mechanisms (NPQ) or by enhancing the rate of carbon assimilation and photosynthetic
28 performance through a process of photochemical quenching (q_p) (Hüner et al. 2012). As a result,
29 photoacclimated plants exhibit a higher maximum photochemical efficiency (F_v/F_m) and increased
30 photosynthetic activity (ϕ_{PSII}) compared to non-acclimated plants. The capacity of the plant to
31 photoacclimate correlates with freezing tolerance (Hüner et al. 1993, Rapacz et al. 2004) and tolerance
32 to high light intensities (Rapacz et al. 2008).

33 In woody species, cold acclimation is a two-step process controlled by a combination of short
34 photoperiod and low temperature, where growth cessation is followed by cold acclimation (Junttila
35 1996). In grasses and herbaceous species, data on impacts of photoperiod on cold hardening are still
36 scarce. Although temperature seems to be the main factor, cold acclimation of grasses is also triggered

1 by photoperiod, especially at higher temperatures (Malyshev et al. 2014). Likewise, hardening of
2 white clover (*Trifolium repens* L.) is enhanced by short photoperiod (Junttila et al. 1990). Recent
3 studies show that the C-repeat binding factor (CBF) cold acclimation pathway in *Arabidopsis thaliana*
4 is regulated by photoperiod (Lee and Thomashow 2012). At higher temperatures, long days caused
5 repression of the CBF pathway, while short days relieved the repression resulting in increased freezing
6 tolerance. This indicates that cold acclimation of herbaceous species is not only controlled by a
7 decreasing temperature, but also photoperiod. Cold acclimation of grasses is also affected by the light
8 intensity (Pollock et al. 1988, Harrison et al. 1997, Höglind et al. 2010), light quality and length of the
9 hardening period (Sjøseth 1964). Winter-hardy cultivars of grasses of northern origin start hardening
10 earlier and achieve a higher freezing tolerance than southern-adapted, less winter-hardy cultivars
11 (Larsen 1994). Longer days stimulate dry matter production in perennial grasses (Hay 1990), and the
12 growth of cultivars adapted to higher northern latitudes are most sensitive to photoperiod (Heide 1982,
13 Solhaug 1991). However, very few studies have focused on temperature \times light interactions during
14 autumn at northern high latitudes. In order to achieve full hardening, active growth in the plants must
15 cease (Rapacz 1998a). The mechanism behind growth cessation of grasses is still poorly understood
16 (Rapacz et al. 2014), but northern-adapted forage grasses seem to have a specific mechanism for
17 growth inhibition during autumn (Østrem et al. 2014).

18 The light regime at northern high latitudes is profoundly different from light regimes at temperate or
19 tropical latitudes (Nilsen 1985). In autumn, the daylength and the global irradiance decrease rapidly
20 with modifying effects of clouds (Fig. 1). The light quality is unique at higher latitudes, with less
21 diurnal alterations, but higher annual variation in the ratio of red and far red light compared to lower
22 latitudes (Nilsen 1985). According to future climate projections, the onset of low positive temperatures
23 required for cold acclimation ($<10^{\circ}\text{C}$) will occur later in the autumn and under considerably shorter
24 daylength and lower irradiance than today (IPCC 2013). It is unknown how higher temperature in
25 combination with reduced irradiance and shorter day length will affect cold acclimation and freezing
26 tolerance of plants in future climate.

27 Timothy (*Phleum pratense* L.) is the most common forage grass species in Northern Norway due to its
28 superior winter survival and good growth at low temperatures and long days. However, when the
29 growing seasons extend into late autumn due to climate changes, perennial ryegrass (*Lolium perenne*
30 L.) may be better adapted in the north than currently. The purpose of this study was to examine the
31 physiological responses of these grasses to a warmer future autumn climate, in order to understand
32 which traits will be important for breeding cultivars for future climatic conditions at higher northern
33 latitudes.

34 We compared photoacclimation, growth rates and freezing tolerance of promising breeding
35 populations of perennial ryegrass and timothy under different combinations of autumn light and
36 temperature in a phytotron at Holt, Tromsø, Norway (69.68°N , 18.94°E). In an earlier study
37 (Dalmannsdottir et al. 2016), we tested the effect of different pre-acclimation temperature treatments

1 on freezing tolerance of the same grass populations and red clover (*Trifolium pratense* L.). In the
2 current experiment, we exposed plants to three successive periods of natural light during autumn,
3 creating three distinct light regimes of progressively lowered irradiance and daylength (Fig. 1). Our
4 hypotheses were; (1) hardening under natural light conditions later in the autumn, at shorter daylength
5 and lower irradiance, reduces the freezing tolerance of perennial ryegrass and timothy; (2) high
6 temperature (12°C) reduces hardening compared to low temperature (6°C), and variable day-time and
7 night-time temperatures (9/3°C), compared with a constant temperature (6°C) affect hardening
8 differently because of diurnal effects; (3) the level of freezing tolerance is regulated by an interaction
9 between temperature and irradiance/daylength; and (4) northern-adapted populations are more
10 sensitive than southern-adapted populations to changing light and temperature conditions.

11

12 **Materials and Methods**

13 **Plant material and growth conditions**

14 Two forage grass species, perennial ryegrass and timothy, were studied. Two populations of each
15 species were included, one selected for the northern regions of Norway (northern-adapted) and the
16 other for the southern regions of Norway (southern-adapted). The perennial ryegrass populations were
17 FuRa9805 (southern-adapted, lat. 55°N (original material) and 61°N (natural selection)) and Fagerlin
18 (northern-adapted, lat. 55°N-63°N, adapted to low winter temperatures), and the timothy populations
19 MTL9701+Grindstad (southern-adapted, lat. 50°N-60°N) and MTV0508-3 (northern-adapted, selected
20 at lat. 59°N/500 m a.s.l. (one generation) and at 67°N (two generations)). For detailed description of
21 populations see Dalmannsdottir et al. (2016).

22 The experiment was conducted in autumn 2012 at Holt, Tromsø (69.68°N, 18.94°E) in phytotron
23 compartments with the temperature controlled to $\pm 0.5^\circ\text{C}$ and the air humidity corresponding to a water
24 vapour deficit of 0.5 kPa. Seedlings were planted in tree nursery trays (60 pots in each tray, one
25 plant/pot, pot size 40 mm diameter x 85 mm height, 45 mm spacing between plants). The pots were
26 filled with fertilised sphagnum peat and perlite (3:1). The plants were watered regularly and fertilised
27 as required with a complete nutrient solution (Hoagland solution, modified from Asher 1978). Apart
28 from the establishment phase, light conditions were natural light during the whole experiment. During
29 establishment, the plants were grown under controlled light conditions in light-isolated chambers for 4
30 weeks at 20°C, 24 h photoperiod. The light source was cool white fluorescent lamps (Philips TLD
31 58W 840), giving $150 \mu\text{mol m}^{-2} \text{s}^{-1}$ photosynthetic photon flux density (PPFD) at plant level (measured
32 with a quantum sensor, Li-1000, Li-Cor) within the range 400-750 nm. During the experimental
33 periods, plants were placed in glasshouse phytotron chambers, allowing natural light from all sides.

34

35 **Experimental design**

36 The whole experiment was conducted under natural light conditions in a phytotron during three
37 separate periods in autumn (year 2012); 5 Sept-10 Oct (early period), 26 Sept -31 Oct (intermediate

1 period) and 17 Oct-21 Nov (late period), resulting in three irradiance/day length treatments here
2 referred to as early, intermediate and late autumn period (Fig. 1). The day length decreased
3 approximately from 14 to 9 h (early period), 11 to 6 h (intermediate period) and 8 to 2 h (late period)
4 during the three periods. The sum of global irradiances for each period decreased from 154, 76 to 21
5 W m^{-2} , respectively. After establishment, similar sized plants were selected for the experiment. Each
6 population was planted in separate trays, which were placed randomly on trolleys within the phytotron
7 compartments. To ensure that plants were at a similar stage and phenology (4 weeks old seedlings)
8 when entering the experiment, we established new seedlings for each successive period. Hence, they
9 were sown at three different dates (8 August, 29 August, 19 September) (Fig. 2).
10 After establishment, plants were subjected to three pre-acclimation temperature treatments, 6, 9/3 (12
11 /12 h) and 12°C, in combination with the three autumn periods (early, intermediate, late) (Fig. 2). The
12 duration of the pre-acclimation temperature treatments was 4 weeks, and thereafter all populations
13 were cold acclimated at 2°C for one week before freezing tests were conducted. The 6°C treatment
14 resembles the current temperature in Sept-Oct in Northern Norway and 12°C an extreme temperature
15 increase in the autumn based on future scenarios until 2050 (Uleberg et al. 2014). We used 6°C and
16 9/3° daytime 08:00 to 20:00/night-time 20:00 to 08:(12h/12h) treatments, giving the same daily
17 temperature sum, to study the influence of changing day and night temperatures on pre-acclimation
18 efficiency and subsequent freezing tolerance levels. Temperature loggers inside the growth chambers
19 secured the accuracy of temperature measurements, but measurements of water vapour deficit were
20 more unstable for the lower temperatures (2 and 6°C), often 30-40% higher than programmed.

21

22 **Morphological measurements**

23 Dry weight of aboveground biomass of 15 plants per population per treatment was recorded at the start
24 of the temperature treatment and at the end of the experiment (Fig. 2), after drying at 60°C for 48 h.
25 The aboveground biomass produced during the experiment was obtained by calculating the difference
26 between the measurements at the start and at the end. Leaf elongation (mm week^{-1}) was measured on
27 15 plants per treatment (in total 180 individuals per autumn period). The youngest emerging leaf on
28 each plant was marked with a thin rubber band and measured weekly during the 5 weeks of pre- and
29 cold acclimation treatments.

30

31 **Chlorophyll fluorescence measurements**

32 The photochemical activity of photosystem II (PSII) was studied by measuring chlorophyll fluorescence
33 (PAM-2500 Portable Chlorophyll Fluorometer; Heinz Walz, Effeltrich, Germany) at room temperature
34 on 15 plants per treatment before and after pre-acclimation treatment, as well as after cold acclimation
35 (Fig. 2) (totally 810 plants for each autumn period), on the same plants as were used to record leaf
36 elongation. The measurements were made on the mid-section of the youngest fully expanded leaves.
37 Before measuring maximum quantum yield of PSII (F_v/F_m) (indication of photoinhibition), leaves were

1 dark-adapted for 15-60 min in leaf clips (8 mm diameter, Walz) and values of F'_m and F_s were recorded
 2 when F_s became stable after re-exposure to actinic red light (800 μmol). Within the same leaf clip, F'_0
 3 was measured after far-red light treatment to ensure rapid opening of PSII reaction centres. Current
 4 quantum yield of PSII (ϕ_{PSII}) (photosynthetic activity indicator) and coefficients of the photochemical
 5 (q_p) and non-photochemical (NPQ) quenching of chlorophyll fluorescence were calculated according to
 6 Genty et al. (1989), Schreiber et al. (1994) and Bilger and Björkman (1991), respectively.

7

8 **Freezing test**

9 At the end of the experiment, freezing tests were performed as described by Pulli et al. (1996) with
 10 modifications (Höglinde et al. 2010) (Fig. 2). Plant roots were washed and single plants were trimmed
 11 to 3 cm top and 1-2 cm root. The crown segments were placed in plastic boxes and covered with fine,
 12 humid sand in a programmable freezer with a temperature sensor in each box. Before freezing
 13 treatments commenced, the temperature was lowered from 2°C to -3°C by 1°C h⁻¹ and kept at -3°C for
 14 13 hours to avoid super-cooling of the plants. The boxes were then frozen to pre-determined
 15 temperatures between -3 and -20°C with 2°C intervals, depending on species and treatment. Freezing
 16 progressed at a cooling rate of -1°C h⁻¹ until -10°C was reached; from then on, the cooling rate was -
 17 3°C h⁻¹ until the predetermined temperature was reached for each treatment. There were two replicate
 18 boxes per predetermined test temperature; each containing 10 crown segments per population per
 19 treatment, i.e. 480 plants per temperature treatment and autumn period summing up to a total number
 20 of 4320 plants. Two boxes per population per treatment were kept at 2°C in darkness as a control.
 21 After freezing, the boxes were placed at 2°C in the dark overnight to thaw, and the crown segments
 22 were transplanted into fertilised peat mixed with perlite. Survival of individual plants was rated as
 23 dead or alive and the LT₅₀ value, i.e. the temperature at which 50% of plants are killed, was estimated
 24 after 3-4 weeks at 20°C and 24 h light (approximately 150 $\mu\text{mol m}^{-2} \text{s}^{-1}$).

25

26 **Statistical analysis**

27 A generalised linear model approach was used to estimate the effects of pre-acclimation temperature
 28 on photosynthetic activity, biomass production and freezing tolerance. Model selection was based on
 29 the Akaike's Information Criteria corrected for small sample sizes (AICc) (Burnham and Anderson,
 30 2002), as this approach is more robust when evaluating interaction effects (Crawley 2007, Gelman and
 31 Hill 2008). For biomass production and photosynthetic activity, a linear model with Gaussian normal
 32 distribution and an identity link was assumed. The full model was defined as *Response* ~
 33 *Treatment*Population*Species*autumn period*, where temperature treatment, population and autumn
 34 period are treated as categorical variables. Four entries were removed as diagnostic plots indicated
 35 them as outliers. Data was log-transformed when variance was heterogeneous (Kleinbaum et al. 1998),
 36 however, untransformed mean values are presented here for clarity. For the freezing test, a logistic
 37 model with logit link function was used. In some of the models there was no overlap of freezing

1 temperature for the group of dead and surviving individuals, hence penalized likelihood was used to
 2 remove bias (library `brglm` in R) (Kosmidis and Firth 2009). The full model for each species was
 3 defined as *Response ~ Treatment*Population*Pre-determined freezing temperature*. We used a non-
 4 linear, three-parameter asymptotic mixed model for estimating leaf elongation (function `nlmer` in the R
 5 library `lme4`). The growth trajectory is described by the function *Leaf length ~ Asym + (R₀-Asym)e^{-e(lrc}
 6 *x week)*, where parameters describe the intercept (*R₀*), the asymptote (*Asym*) and the logistic rate constant
 7 (*lrc*) (Crawley 2007). Here, the *R₀*-value describes leaf length at the start of the experiment and the
 8 *Asym* value describes the leaf length at cessation of growth. While the *lrc*-value describes the logistic
 9 growth rate constant, the absolute growth rate at a certain time is given by the combination of the
 10 parameters as described by the non-linear function above. Individual plant identity was included as a
 11 random term to avoid pseudoreplication. As we were interested in main effects and the effects sizes,
 12 we chose to evaluate differences in leaf elongation by comparing 95% confidence intervals rather than
 13 multiple comparison approaches (Saville 2015, Garcia 2004, Rothman 1990). For non-linear mixed
 14 models, the confidence interval was approximated by mean ± 2 x SE (Gelman and Hill 2008).
 15 Predictors were considered significant if their 95% confidence interval did not include zero. All
 16 statistical analyses were performed using R (R version 3.0.1) and Minitab 16 (Minitab Inc. 2010, State
 17 College, PA, USA). Model comparisons and population statistics are presented in supplementary table
 18 S2-15.*

19

20 **Results**

21 **Biomass production**

22 Total biomass production decreased gradually from the early autumn period to the late period.
 23 However, the decrease was also dependent on temperature (Table 1). During the early autumn period,
 24 markedly more biomass was produced at 12 compared to 6 and 9/3°C (Table 1). Southern-adapted
 25 populations also produced more biomass at 9/3 compared to 6°C during this period. During the
 26 intermediate autumn period, plants at 12 and 9/3°C produced more biomass than plants at 6°C, though
 27 not significant for the northern-adapted population of perennial ryegrass (Table 1). During the late
 28 autumn period, there were no significant differences in biomass production between temperature
 29 treatments (Table S3). We found no consistent differences in biomass production between northern
 30 and southern-adapted populations (Table S3). However, in particular northern-adapted perennial
 31 ryegrass produced more biomass than timothy at 6°C and during the early autumn period.

32

33 **Leaf elongation**

34 The effect of autumn period on leaf elongation was dependent on the temperature treatment and
 35 population. Leaf elongation rate was always higher at 12 than at 6 and 9/3°C for all autumn periods
 36 and populations (reflected in higher *lrc* value, Fig. 3, Table S1). The effect of temperature on leaf
 37 elongation was more pronounced at the late autumn period in particular for northern perennial ryegrass

1 (Fig. 3, Table S1). Leaf elongation of plants at 12°C was faster at later autumn periods compared to
 2 earlier periods (Fig. 3, Table S1), though not significantly different for the southern-adapted
 3 population of perennial ryegrass at the early and intermediate autumn periods. In the late autumn
 4 period, leaves ceased growth at lower leaf length at 6°C than at 12 or 9/3°C resulting in longer leaves
 5 at 12 and 9/3°C (reflected in lower asymptote values of leaf length at 6°C, Table S1). Only the
 6 southern-adapted population of perennial ryegrass showed no difference in elongation rate between
 7 treatments at 6 and 9/3°C in the early period. During the intermediate autumn period, all populations
 8 grew significantly faster at 9/3°C compared to 6°C. In the late autumn period, the same effect was
 9 observed in perennial ryegrass, but was not significant in the timothy populations. There was no
 10 general difference between southern and northern-adapted populations regarding leaf elongation rate.

11

12 **Chlorophyll fluorescence**

13 The photochemical activity measured after pre-acclimation temperature treatments is presented in Fig.
 14 4. The photochemical efficiency (F_v/F_m) were overall higher in the intermediate autumn period than in
 15 the early and late autumn periods (Fig. 4, Table S8). In the early autumn period, F_v/F_m increased at
 16 higher temperatures, except for plants of the southern-adapted timothy population (Fig. 4, Table S8).
 17 Opposite, in the late autumn period, F_v/F_m values decreased at increasing temperatures. The non-
 18 photochemical quenching (NPQ) values were highest in the intermediate autumn period for both
 19 species. In timothy, plants at 12°C had the lowest and plants at 6°C the highest NPQ values at both
 20 late and intermediate autumn periods (Fig. 4, Table S9), whereas this was not evident in the early
 21 autumn period. This temperature effect was not observed in the northern-adapted and only in the late
 22 autumn period in the southern-adapted perennial ryegrass.

23 Photosynthetic activity (ϕ_{PSII}) was affected mainly by autumn period; it decreased with later autumn
 24 periods and increasing temperature (Fig. 4, Table S10). Photochemical quenching (q_p) showed similar
 25 trends as ϕ_{PSII} (Fig. 4, Table S11). Photochemical activity before pre-acclimation treatments was not
 26 significantly different between populations and treatments, with the exception of F_v/F_m which varied
 27 slightly but not consistently between populations and treatments (data not shown). After pre-
 28 acclimation, F_v/F_m and NPQ values increased for all temperature treatments and autumn periods
 29 compared to before pre-acclimation, as observed by Dalmannsdottir et al. (2016). Also, q_p and ϕ_{PSII}
 30 values increased after pre-acclimation, except at the late autumn period when values before pre-
 31 acclimation were higher. The only change in photochemical activity after cold acclimation at 2°C was
 32 a slight increase in F_v/F_m values at the early autumn period, especially at lower temperature (data not
 33 shown).

34 No significant differences were found between northern- and southern-adapted populations regarding
 35 photochemical activity.

36 **Freezing tolerance**

1 Plants pre-acclimated in the late autumn period and at the highest temperature displayed lowest
2 freezing tolerance irrespective of species and population (Table 2, Fig. 5). Plants pre-acclimated at
3 12°C were less freezing tolerant than plants acclimated at 6 and 9/3°C. There were no significant
4 differences in freezing tolerance between the 6 and 9/3°C treatments (Table S14, S15). There was no
5 significant differences between plants pre-acclimated in the early and the intermediate autumn period.
6 Northern-adapted populations had higher freezing tolerance compared to southern ones, except for
7 those pre-acclimated in the late autumn period and at the highest acclimation temperature (Fig. 5,
8 Table 2). In the intermediate and the late autumn period freezing tolerance was more strongly reduced
9 by the 12 than the 6°C temperature treatment in northern-adapted populations compared to southern-
10 adapted (Fig. 5, Table 2).

11

12 **Discussion**

13 We found that interactions between temperature and day length/irradiance had strong effects on
14 growth, cold acclimation and freezing tolerance of perennial ryegrass and timothy populations with
15 diverse adaptations. A combination of low irradiance/short day length and higher than normal
16 temperatures, a scenario expected with global warming at higher latitudes, reduced freezing tolerance
17 and photosynthetic activity substantially in all populations. The northern-adapted populations
18 generally had higher freezing tolerance than the southern-adapted, but not at the combination of
19 shortest day length and highest temperature. This indicates that populations adapted at higher latitudes
20 are vulnerable to the predicted climate changes, which will be most pronounced in these regions.

21

22 **Temperature and daylength/irradiance effects on growth**

23 All populations responded to a lower irradiance and shorter daylength with reduced dry-matter
24 production. This is in accordance with previous studies of timothy and other high latitude grass species
25 (Heide et al. 1985, Solhaug 1991, Wu et al. 2004). In our study, leaf elongation rate was similar (at
26 6°C) or increased (9/3 and 12°C) at later autumn periods. At later autumn periods, the plants
27 (especially timothy) were suffering from low turgor pressure despite normal soil humidity (data not
28 shown). Leaves of timothy were thin and etiolated, while leaves of perennial ryegrass were narrow but
29 with more turgor than timothy, especially plants of northern-adapted perennial ryegrass. Etiolated
30 growth of grasses is a well-known response to limited light conditions (Robson et al. 1988). Peri et al.
31 (2007) also found that etiolated pastures with cocksfoot produced less dry matter. Schnyder and
32 Nelson (1988) found that leaf elongation in tall fescue (*Festuca arundinacea*) was up to 65% faster
33 during the dark period during the diurnal cycle, depending on the light intensity and temperature shift.
34 Leaf elongation in grasses is known to increase at higher temperatures, and this was confirmed in the
35 present study and in a previous study where the same populations were tested for temperature
36 responses (Dalmannsdottir et al. 2016). Different day/night temperature (9/3°C) stimulated biomass

1 production and leaf elongation compared to the corresponding constant temperature (6°C) in the early
2 and intermediate autumn period, especially in southern-adapted populations. During the late autumn
3 period, the light level was a limiting factor, thus there were no temperature effects on biomass
4 production. However, leaf elongation was stimulated at 9/3 but not at 6°C, as a response to lower
5 irradiance and shorter day length. Junttila (1985) found that shoot elongation of timothy cultivars was
6 stimulated by alternating temperatures compared to corresponding constant temperatures, possibly
7 related to light × temperature interactions. The same effect has been shown in pea (*Pisum sativum*)
8 (Grindal et al. 1998) and oilseed rape (*Brassica napus* L. var. *oleifera*) (Rapacz 1998b).

9 Under low temperature and long day conditions, it has been shown that dry matter production is
10 generally more strongly stimulated in grass cultivars adapted to northern high latitudes compared to
11 ecotypes from lower latitudes (Solhaug 1991, Østgård and Eagles 1971). Our results did not show
12 significant differences in biomass production between northern and southern-adapted populations,
13 except for the northern-adapted population of perennial ryegrass (cv. ‘Fagerlin’), which produced
14 more dry matter at 6°C and during the early autumn period compared to the southern-adapted.
15 Furthermore, at the late autumn period, we observed that the northern-adapted populations of both
16 species had a more compact growth habit than the southern-adapted. The northern-adapted perennial
17 ryegrass, cv. ‘Fagerlin’, showed relatively high photosynthetic activity and reduced leaf elongation
18 growth during autumn in a field study in Norway (Østrem et al. 2014). It had good winter survival and
19 high plot coverage the following spring. Together with our findings this indicates that cv. ‘Fagerlin’
20 may be able to utilize a prolonged growth season without sacrificing the level of freezing tolerance.
21 This cultivar may thus be a promising germplasm resource for future breeding programs.

22

23 **Temperature and daylength effects on photoacclimation**

24 Cold acclimation is known to increase photosynthetic performances (Yamasaki et al. 2002; Hüner et
25 al. 2014), which results in higher PSII photosynthetic activity at lower temperatures (Dalmannsdottir
26 et al. 2016). This is supported by our study especially under early autumn light conditions.
27 Photosynthetic activity was more affected by autumn period than temperature, and light conditions
28 during later autumn periods reduced the photosynthetic activity in all populations. Increasing q_p
29 (photochemical quenching) with increasing irradiance/day length and decreasing temperature shows
30 that the photochemical acclimation mechanism was more predominant than the non-photochemical
31 mechanism, as demonstrated before in a response to temperature in studies with winter rye (Huner
32 1985) and oilseed rape (Rapacz and Janowiak 1998). Our results did not indicate an active NPQ
33 mechanism, but the NPQ values observed in timothy at the two later autumn periods may be caused by
34 etiolation of leaves rather than temperature. In etiolated leaves and leaves at low light intensities, the
35 amount of active PSII reaction centres is reduced (Miyata et al. 2012) as an adaptive response to

1 protect the photosystem (Tikkanen et al. 2014), resulting in lower NPQ values. The higher sensitivity
2 of timothy compared to perennial ryegrass in relation to leaf etiolation is reflected in the fluorescence
3 measurements. NPQ mechanisms have been found to dissipate excess light during cold acclimation in
4 winter hardy grass species (Humphreys et al. 2007) and northern-adapted cultivars (Rapacz et al.
5 2004). In our study, southern and northern-adapted populations were not different as regards q_p
6 mechanisms. A slightly higher ϕ_{PSII} at 6 compared to 9/3°C indicates lower excitation pressure in
7 plants at 6°C because of lower temperature during the daylight period. Photoinhibition was observed
8 at the early autumn period in combination with low temperature. A shift from the early autumn period
9 (5 Sept -10 Oct) to the intermediate (26 Sept - 31 Oct) reduced the damages of PSII, expressed as
10 higher F_v/F_m values. However, reduction of the autumn light conditions during the late autumn period
11 decreased the F_v/F_m values again, probably because the irradiance was below a critical limit for the
12 induction of photoacclimation. There was a slight increase in F_v/F_m during cold acclimation at 2°C in
13 the early autumn period (data not shown). This indicates that the photochemical mechanism of
14 photoacclimation, which was induced during pre-acclimation, further increased the tolerance to cold-
15 induced photoinhibition during cold acclimation.

16

17 **Temperature and daylength effect on freezing tolerance**

18 The treatments under the late autumn period and highest acclimation temperature (12°C) had the
19 lowest freezing tolerance for both species and populations. We have shown that a rise in pre-
20 acclimation temperature (9, 12, 15°C) under controlled light conditions decreased both cold
21 acclimation capacity and photoacclimation in the same populations (Dalmannsdottir et al. 2016).
22 Malyshev et al. (2014) found temperature to be a stronger trigger of cold acclimation than photoperiod
23 in an experiment with the grass species *Arrhenatherum elatius*. In the current study, northern-adapted
24 populations had higher freezing tolerances than southern-adapted except at the shortest photoperiod
25 and the highest temperature where there were no differences in freezing tolerance. This indicates that
26 today's northern-adapted breeding material may lose its advantages over southern-adapted in the
27 future climate.

28 Freezing tolerance was reduced in plants at the late autumn period compared to the early and the
29 intermediate period. Treatment at later autumn light conditions includes reduction in the total
30 irradiation energy, and higher light intensity or irradiance is known to increase cold acclimation in
31 perennial ryegrass (Pollock et al. 1988, Harrison et al. 1997). Light intensity is even more important
32 for cold acclimation than photoperiod (Lawrence et al. 1973). In a pilot study in autumn 2011, the
33 freezing tolerance of timothy cv. 'Grindstad' at 6°C, grown under natural light at the same periods as
34 used in the current study, was tested. Plants in the pilot study expressed gradually lower freezing
35 tolerance when acclimated at later autumn light conditions (data not shown), significantly different

1 between all three autumn periods. In the current study, there were no differences between the early and
2 the intermediate autumn period regarding freezing tolerance. The plant populations were not exactly
3 the same as in the pilot study, and more importantly, yearly fluctuations in the amounts of clouds
4 cause differences in irradiance and affect the cold acclimation process. Since we tested effects of
5 natural light conditions during autumn, the effects of irradiance and day length are confounded and
6 cannot be separated in this study. It is likely that the reduction in irradiance is even more important
7 than short days in relation to reduced freezing tolerance of the populations.

8 We did not find any significant difference in freezing tolerance between 6 and 9/3°C. Studies of
9 Sjøseth (1971) support these findings, but Eagles and Williams (1992) found that high day and low
10 night temperatures (10/2°C) gave a positive effect on freezing tolerance of perennial ryegrass
11 compared to a constant temperature (10°C). The effect of diurnal temperature differences on cold
12 acclimation seems to be a complicated interaction between day length, light quality and intensity.

13 Timothy is known to be considerably more winter-hardy than perennial ryegrass (Sjøseth 1971,
14 Jørgensen et al. 2010), but this was not the case in the present study, possibly because plants did not
15 reach maximum seasonal hardening after only five weeks of acclimation treatments. On the other
16 hand, the freezing tolerance capacity *per se* does not seem to be the limiting factor for poor survival of
17 perennial ryegrass at in northern high latitude areas. Other factors involved in seasonal adaptation like
18 inadequate growth cessation (Østrem et al. 2014), low non-structural carbohydrate accumulation
19 during winter (Østrem et al. 2011), low resistance to ice encasement (Höglind et al. 2010) and
20 susceptibility to fungal diseases (Hofgaard et al. 2003) may contribute more to the poor winter
21 survival than freezing tolerance.

22 Photoacclimation processes responded more strongly to photoperiod than to temperature whereas
23 freezing tolerance responded more to temperature than photoperiod. Both photoacclimation
24 (photochemical quenching) and freezing tolerance was reduced with decreasing autumn light
25 conditions and increasing temperature. In studies by Rapacz et al. (2004), winter survival of
26 *Festulolium* genotypes correlated with increased energy dissipation and lower photosynthetic activity
27 of PSII before winter.

28 Our results indicate that the projected climate change in the north may reduce freezing tolerance in
29 grasses because plants will be pre-acclimated at higher temperatures and shorter day length. Current
30 adapted breeding populations may have unacceptable freezing tolerance in future climate. The present
31 species and cultivars may therefore have to be replaced by species and cultivars, which are able to
32 acclimate adequately under new day length × temperature combinations, combinations which are
33 unique in the global context. Future breeding programs for northern high-latitude areas will need

1 adapted germplasm and introgression of southern-adapted material in order to produce high yielding
2 and persistent grass cultivars adapted to the future climates.

3 **Author contributions**

4 Dalmannsdottir, Jørgensen, Rapacz and Rognli designed, guided or participated in performing the
5 experiment. Dalmannsdottir wrote the first draft and corrected the manuscript. Østrem and Larsen
6 provided the plant material. Rødven did most statistical analysis and wrote the chapter on statistical
7 analysis. All co-authors discussed results, reviewed and corrected the manuscript.

8
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16 **References**

- 17 Asher CJ. 1978. Natural and synthetic culture media for spermatophytes. In: Rechcigal M Jr., (Ed.),
18 Handbook of Nutrition and Food, Section G: Diets, culture media, food supplements. Vol. III.
19 Culture media for microorganisms and plants, CRC Press, Cleveland, Ohio, pp 575-609
- 20 Bertrand A, Castonguay Y (2003) Plant adaptations to overwintering stresses and implications of
21 climate change. *Can J Bot* 81: 1145-1152
- 22 Bilger W, Björkman O (1991) Temperature dependence of violaxanthin de-epoxidation and non-
23 photochemical fluorescence quenching in intact leaves of *Gossypium hirsutum* L. and *Malva*
24 *perviflora* L. *Planta* 184: 226-234
- 25 Burnham KP, Anderson DR (2002) Model selection and multimodel inference: A practical
26 information-theoretical approach, 2nd edn. Springer-Verlag, New York, 488 p
- 27 Chinnusamy V, Zhu J, Zhu J-K (2006) Gene regulation during cold acclimation in plants. *Physiol*
28 *Plant* 126: 52-61
- 29 Cooper EJ (2014) Warmer shorter winters disrupt arctic terrestrial ecosystems. *Annu Rev Ecol Evol*
30 *Syst* 45: 271-295
- 31 Crawley MJ (2007) The R book, 1st. edn. John Wiley & Sons, Ltd., West Sussex, pp 1-942
- 32 Dalmannsdottir S, Rapacz M, Jørgensen M, Østrem L, Larsen A, Rødven R, Rognli OA (2016)
33 Temperature before cold acclimation affects cold tolerance and photoacclimation in timothy
34 (*Phleum pratense* L.), perennial ryegrass (*Lolium perenne* L.) and red clover (*Trifolium pratense*
35 L.). *J Agron Crop Sci* 202: 320-330

- 1 Eagles CF, Williams J (1992) Hardening and dehardening of *Lolium perenne* in response to
2 fluctuating temperatures. *Ann Bot* 70: 333-338
- 3 Ensminger I, Busch F, Huner NPA (2006) Photostasis and cold acclimation: sensing low temperature
4 through photosynthesis. *Physiol Plant* 126: 28-44
- 5 Garcia LV (2004) Escaping the Boferroni iron claw in ecological studies. *Oikos* 105(3): 657-663
- 6 Gelman A, Hill J (2008) Data analysis using regression and multilevel/hierarchical models. –
7 Analytical methods for social research. Cambridge University Press, Cambridge, 652 p
- 8 Genty B, Briantais J-M, Baker NR (1989) The relationship between the quantum yield of
9 photosynthetic electron transport and quenching of chlorophyll fluorescence. *Biochim Biophys*
10 *Acta* 990: 87-92
- 11 Gray GR, Chauvin LP, Sarhan F, Huner NPA (1997) Cold acclimation and freezing tolerance (A
12 complex interaction of light and temperature). *Plant Physiol* 114: 467-474
- 13 Grindal G, Junttila O, Reid JB, Moe R (1998) The response to gibberellin in *Pisum sativum* grown
14 under alternating day and night temperature. *J Plant Growth Regul* 17: 161-167
- 15 Harrison J, Tonkinson C, Eagles C, Foyer C (1997) Acclimation to freezing temperatures in perennial
16 ryegrass (*Lolium perenne*). *Acta Physiol Plant* 19: 505-511
- 17 Hay RKM (1990) The influence of photoperiod on the dry-matter production of grasses and cereals.
18 *New Phytol* 116: 233-254
- 19 Heide OM (1982) Effects of photoperiod and temperature on growth and flowering in Norwegian and
20 British timothy cultivars (*Phleum pratense* L.). *Acta Agric Scand Sect B - Plant Soil Sci* 32: 241–
21 252
- 22 Heide OM, Hay RKM, Bauergröd H (1985) Specific daylength effects on leaf growth and dry-matter
23 production in high-latitude grasses. *Ann Bot* 55: 579-586
- 24 Hofgaard IS, Vollsnes AV, Marum P, Larsen A, Tronsmo AM (2003) Variation in resistance to
25 different winter stress factors within full-sib family of perennial ryegrass. *Euphytica* 134: 61-75
- 26 Höglind M, Bakken AK, Jørgensen M, Østrem L (2010) Tolerance to frost and ice encasement in
27 cultivars of timothy and perennial ryegrass during winter. *Grass Forage Sci* 65: 431-445
- 28 Humphreys MW, Gasior D, Lesniewska-Bocianowska A, Rapacz M (2007) Androgenesis as a means
29 of dissecting complex genetic and physiological controls: selecting useful gene combinations for
30 breeding freezing tolerant grasses. *Euphytica* 158: 337-345
- 31 Huner NPA (1985) Acclimation of winter rye to cold-hardening temperatures results in an increased
32 capacity for photosynthetic electron transport. *Can J Bot* 63: 506-511
- 33 Hüner NPA, Öquist G, Hurry VM, Kroll M, Falk S, Griffith M (1993) Photoynthesis, photoinhibition
34 and low temperature acclimation in cold tolerant plants. *Photosynth Res* 37: 19-39
- 35 Hüner NPA, Bode R, Dahal K, Hollis L, Rosso D, Krol M, Ivanov AG (2012) Chloroplast redox
36 imbalance governs phenotypic plasticity: the "grand design of photosynthesis" revisited. *Front*
37 *Plant Sci* 3: 255. doi:10.3389/fpls.2012.00255

- 1 Hüner NPA, Bode R, Dahal K, Busch FA, Possmayer M, Szyszka B, Rosso D, Ensminger I, Krol M,
2 Ivanov AG, Maxwell DP (2013) Shedding some light on cold acclimation, cold adaptation, and
3 phenotypic plasticity. *Botany* 91: 127-136
- 4 Hüner NPA, Dahal K, Kurepin L, Savitch L, Singh J, Ivanov AG, Kane K, Sarhan F (2014) Potential
5 for increased photosynthetic performance and crop productivity in response to climate change:
6 role of CBFs and gibberellic acid. *Front Chem* 2: 18. doi: 10.3389/fchem.2014.00018
- 7 IPCC (2013) Climate Change 2013. The Physical Science Basis. Contribution of Working Group I to
8 the Fifth Assessment Report of the Intergovernmental Panel on Climate Change [Stocker, T.F., D.
9 Qin, G.-K. Plattner, M. Tignor, S.K. Allen, J. Boschung, A. Nauels, Y. Xia, V. Bex and P.M.
10 Midgley (eds.)]. Cambridge University Press, Cambridge, United Kingdom and New York, pp
11 1535. doi:10.1017/CBO9781107415324
- 12 Jørgensen M, Østrem L, Höglind M (2010) De-hardening in contrasting cultivars of timothy and
13 perennial ryegrass during winter and spring. *Grass Forage Sci* 65: 38-48
- 14 Junttila O (1985) Experimental control of flowering and vivipary in timothy (*Phleum pratense*). *Physiol*
15 *Plant* 63: 35-42
- 16 Junttila, O (1996) Plant adaptation to temperature and photoperiod. *Agr Food Sci Finland* 5: 251-260
- 17 Junttila O, Svenning MM, Solheim B (1990) Effects of temperature and photoperiod on frost
18 resistance of white clover (*Trifolium repens*) ecotypes. *Physiol Plant* 79: 427-438
- 19 Keily J, MacGregor DR, Smith RW, Millar AJ, Halliday KJ, Penfield S (2013) Model selection
20 reveals control of cold signalling by evening-phased components of the plant circadian clock. *Plant*
21 *J* 76: 247-257
- 22 Kleinbaum DG, Kupper LL, Muller KE, Nizam A (1998) *Applied Regression Analysis and Other*
23 *Multivaivable Methods*, 3rd Edn. Pacific Grove, Duxbury Press pp. 1-798
- 24 Kosmidis I, Firth D (2009) Bias reduction in exponential family nonlinear models. *Biometrika* 96: 793–804
- 25 Larsen A (1994) Breeding winter hardy grasses. *Euphytica* 77: 231-237
- 26 Lawrence T, Cooper JP, Breese EL (1973) Cold tolerance and winter hardiness in *Lolium perenne*: II.
27 Influence of light and temperature during growth and hardening. *J Agric Sci* 80: 341-348
- 28 Lee C-M, Thomashow MF (2012) Photoperiodic regulation of the C-repeat binding factor (CBF) cold
29 acclimation pathway and freezing tolerance in *Arabidopsis thaliana*. *PNAS* 109: 15054-15059
- 30 Malyshev AV, Henry HAL, Kreyling J (2014) Relative effects of temperature vs. photoperiod on
31 growth and cold acclimation of northern and southern ecotypes of the grass *Arrhenatherum*
32 *elatius*. *Environ Exp Bot* 106: 189-196
- 33 Miyata K, Noguchi K, Terashima I (2012) Cost and benefit of the repair of photodamaged
34 photosystem II in spinach leaves: roles of acclimation to growth light. *Photosynth Res* 113: 165-
35 180
- 36 Miura K, Furumoto T (2013) Cold signaling and cold response in plants. *Int J Mol Sci* 14: 5312-5337.

- 1 Nilsen J (1985) Light climate in northern areas. In: Junttila O, Kauren Å (eds) Plant production in the
2 north. Norwegian University Press, Oslo pp 62-72
- 3 Palva ET, Thtiharju S, Tamminen I, Puhakainen T, Laitinen R, Svenson J, Helenius E, Heino P (2002)
4 Biological mechanism of low temperature stress response: Cold acclimation and development of
5 freezing tolerance in plants. In: Iwanaga M (compiled by) Genetic Engineering of Crop Plants for
6 Abiotic Stress JIRCAS Working Report 23, 2002, pp 9-15
- 7 Peri PL, Lucas RJ, Moot DJ (2007) Dry matter production, morphology and nutritive value of *Dactylis*
8 *glomerata* growing under different light regimes. *Agroforest Syst* 70: 63-79
- 9 Pollock CJ, Eagles CF, Sims IM (1988) Effect of photoperiod and irradiance changes upon
10 development of freezing tolerance and accumulation of soluble carbohydrate in seedlings of *Lolium*
11 *perenne* grown at 2°C. *Ann Bot* 62: 95-100
- 12 Pulli S, Hjortsholm K, Larsen A, Gudleifsson B, Larsson S, Kristiansson B, Hoemmoe L, Tronsmo
13 AM, Ruuth P, Kristiansson C (1996) Development and evaluation of laboratory testing methods
14 for winter hardiness breeding. Nordic Gene Bank publication 32, Alnarp Sweden, 1996, 68 p
- 15 Quellet F, Charron J-B (2013) Cold acclimation and freezing tolerance in plants. In: eLS. John Wiley
16 & Sons, Ltd: Chichester. DOI: 10.1002/9780470015902.a0020093.pub2
- 17 Rapacz M (1998a) Physiological effects of winter rape (*Brassica napus* var *oleifera*) prehardening to
18 frost. II. Growth, energy partitioning and water status during cold acclimation. *J Agron Crop Sci*
19 181: 81-87
- 20 Rapacz M (1998b) The after-effects of temperature and irradiance during early growth of winter
21 oilseed rape (*Brassica napus* L. var. *oleifera*, cv. Góczyński) seedlings on the progress of their cold
22 acclimation. *Acta Physiol Plant* 20: 73-78
- 23 Rapacz M, Janowiak F (1998) Physiological effects of winter rape (*Brassica napus* var *oleifera*)
24 prehardening to frost. I. Frost resistance and photosynthesis during cold acclimation. *J Agron Crop*
25 *Sci* 181: 13-20
- 26 Rapacz M, Gąsior D, Zwierzykowski Z, Leśniewska-Bocianowska A, Humphreys HW, Gay AP
27 (2004) Changes in cold tolerance and the mechanisms of acclimation of photosystem II to cold
28 hardening generated by anther culture of *Festuca pratensis* × *Lolium multiflorum* cultivars. *New*
29 *Phytol* 161: 105-114
- 30 Rapacz M, Wolanin B, Hura K, Tyrka M (2008) The effects of cold acclimation on photosynthetic
31 apparatus and the expression of COR14b in four genotypes of barley (*Hordeum vulgare*)
32 contrasting in their tolerance to freezing and high-light treatment in cold conditions. *Ann Bot* 101:
33 689-699
- 34 Rapacz M, Ergon Å, Höglind M, Jørgensen M, Jurczyk B, Østrem L, Rognli OA, Tronsmo AM (2014)
35 Overwintering of herbaceous plants in a changing climate. Still more questions than answers. *Plant*
36 *Sci* 225: 34-44

- 1 Robson MJ, Ryle GJA, Woledge J (1988) The grass plant – its form and function. In: Jones MB,
2 Lazenby A (eds) The grass crop – The physiological basis of production. Chapman and Hall, New
3 York pp 25-73
- 4 Rothman KJ (1990) No Adjustments Are Needed for Multiple Comparisons. *Epidemiology* 1(1): 43-
5 46
- 6 Sandve SR, Kosmala A, Rudi H, Fjellheim S, Rapacz M, Yamada T, Rognli OA (2011) Molecular
7 mechanisms underlying frost tolerance in perennial grasses adapted to cold climates. *Plant Sci*
8 180: 69-77
- 9 Saville DJ (2015) Multiple comparison procedures - cutting the gordian knot. *Agron J* 107(2): 730-735
- 10 Schreiber U, Bilger W. and Neubauer C (1994) Chlorophyll fluorescence as a noninvasive indicator
11 for rapid assessment of in vivo photosynthesis. In: Schultze E-D, Caldwell MM (eds)
12 Ecophysiology of photosynthesis. Germany Springer-Verlag, Berlin, pp 49-70
- 13 Schnyder H, Nelson CJ (1988) Diurnal growth of tall fescue leaf blades. *Plant Physiol* 86: 1070-1076
- 14 Sjöseth H (1964) Studies on frost hardening in plants. *Acta Agr Scand* XIV:178-192
- 15 Sjöseth H (1971) Winter hardiness in different meadow and pasture plants. *Meldinger fra Norges*
16 *Landbrukshøgskole* 50: 1-39
- 17 Solhaug KA (1991) Influence of photoperiod and temperature on dry matter production and
18 chlorophyll content in temperate grasses. *Norw J Agr Sci* 5: 365-384
- 19 Tomashow MF (1999) Plant cold acclimation: freezing tolerance genes and regulatory mechanisms.
20 *Annu Rev Plant Phys* 50: 571-599
- 21 Tikkanen M, Mekala NR, Aro E-M (2014) Photosystem II photoinhibition-repair cycle protects
22 photosystem I from irreversible damage. *Biochim Biophys Acta* 1837: 210-215
- 23 Uleberg E, Hanssen-Bauer I, van Oort B, Dalmannsdottir S (2014) Impact of climate change on
24 agriculture in Northern Norway and potential strategies for adaptation. *Clim Change* 122: 27-39
- 25 Wingler A (2015) Comparison of signaling interactions determining annual and perennial plant
26 growth in response to low temperature. *Front Plant Sci* 5: 794. doi: 10.3389/fpls.2014.00794
- 27 Wu Z, Skjelvåg AO, Baadshaug OH (2004) Quantification of Photoperiodic effects on growth of
28 *Phleum pratense*. *Ann Bot* 94: 535-543
- 29 Yamasaki T, Yamakawa T, Yamane Y, Koike H, Satoh K, Katoh S (2002) Temperature acclimation of
30 photosynthesis and related changes in photosystem II electron transport in winter wheat. *Plant*
31 *Physiol* 128: 1087-1097
- 32 Yoshida M, Abe J, Moriyama M, Shimokawa S, Nakamura Y (1997) Seasonal changes in the physical
33 state of crown water associated with freezing tolerance in winter wheat. *Physiol Plant* 99: 363-370
- 34 Østgård O, Eagles CF (1971) Variation in growth and development in natural populations of *Dactylis*
35 *glomerata* from Norway and Portugal. II. Leaf Development and Tillering. *J Appl Ecol* 8: 383-391

- 1 Østrem L, Rapacz M, Jørgensen M, Höglind M (2011) Effect of developmental stage on carbohydrate
- 2 accumulation patterns during winter of timothy and perennial ryegrass. *Acta Agric Scand Sect B –*
- 3 *Plant Soil Sci* 61: 153-163
- 4 Østrem L, Rapacz M, Larsen A, Dalmanndottir S, Jørgensen M (2014) Influences of growth cessation
- 5 and photoacclimation on winter survival of non-native *Lolium-Festuca* grasses in high-latitude
- 6 regions. *Environ Exp Bot* 111: 21-31
- 7

1 **Fig. 1.** Experimental setup including global irradiation (Wm^{-2}) (solid line) during experimental period
2 in year 2012. A sum of radiation for each light period during autumn (early, intermediate, and late) is
3 presented. Day length (hours) in Tromsø (dotted line).

4

5 **Fig. 2.** Timeline for measurements. Time points for measurement of; (a) above ground biomass, (b)
6 chlorophyll fluorescence and (c) freezing tolerance. Leaf elongation was measured every week during
7 pre- and cold acclimation treatments. Plants were propagated from seeds at 20°C under 24 h
8 photoperiod and $150 \mu\text{mol m}^{-2} \text{s}^{-1}$ artificial light. Pre-acclimation treatments were 6, $9/3^{\circ}\text{C}$ (12 h
9 day/12 h night) and 12°C under natural light. Cold acclimation (C) at 2°C was carried out under
10 natural light conditions.

11

12 **Fig. 3.** Estimated values of leaf elongation (mm) for perennial ryegrass (PRG) and timothy measured
13 every week during 4 weeks of treatment with pre-acclimation temperatures 6, $9/3^{\circ}\text{C}$ (12 h day/12 h
14 night) and 12°C , and 1 week of treatment with cold acclimation at 2°C for early, intermediate and late
15 autumn period. Real value raw data included, 12°C (cross), $9/3^{\circ}\text{C}$ (triangle), 6°C (circle).

16

17 **Fig. 4.** Changes in fluorescence parameters in southern and northern-adapted populations of perennial
18 ryegrass and timothy measured after the three different pre-acclimation temperature treatments.
19 Estimated mean values with 95% confidence intervals for full parametric model are presented.
20 E=Early period, I=intermediate period, L=Late period

21

22 **Fig. 5.** Survival of plant populations as a function of freezing temperature ($^{\circ}\text{C}$) in a freezing test at the
23 end of the experiment. The line at 50% survival indicates the LT_{50} value for the population. Predicted
24 values are presented for each temperature treatment (6, $9/3$, 12°C) and each light period during autumn
25 (early, intermediate, late). Empirical proportions of survival at different freezing temperature are shown
26 with dots, 12°C (cross), $9/3^{\circ}\text{C}$ (triangle), 6°C (circle).