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Some like it wet – biological characteristics underpinning tolerance of extreme water stress events in Antarctic bryophytes.

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Keywords

desiccation, submergence, chlorophyll fluorescence, ?13C, fatty acids, soluble carbohydrates, climate change, Antarctica

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Some like it wet – biological characteristics underpinning tolerance of extreme water stress events in Antarctic bryophytes.

ANTARCTIC MOSS AND EXTREME WATER STRESS

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Key words: desiccation, submergence, chlorophyll fluorescence, $\delta^{13}C$, fatty acids, soluble carbohydrates, climate change, Antarctica.

Abbreviations: ASPA: Antarctic Specially Protected Area, CWC: critical water content, CRT: critical recovery time, F_v/F_m: maximum quantum yield of PSII, MUFA: monounsaturated fatty acids ΦPSII; effective quantum yield of PSII, PUFA: polyunsaturated fatty acids, SFA: saturated fatty acids, TWC: turf water content, UFA: unsaturated fatty acid.

Abstract

Antarctic bryophyte communities presently tolerate physiological extremes in water availability, surviving both desiccation and submergence events. This study investigated the relative ability of three Antarctic moss species to tolerate physiological extremes in water availability and identified physiological, morphological, and biochemical characteristics that assist species performance under such conditions. Tolerance of desiccation and submergence was investigated using chlorophyll fluorescence during a series of field- and laboratory-based water stress events. Turf water retention and degree of natural habitat submergence were determined from gametophyte shoot size and density and $\delta^{13}C$ signatures respectively. Finally, compounds likely to assist membrane structure and function during desiccation events (fatty acids and soluble carbohydrates) were determined. The results of this study show significant differences in the performance of the three study species under contrasting water stress events. The results indicate that the three study species occupy distinctly different ecological niches with respect to water relations and provide a physiological explanation for present species distributions. The poor tolerance of submergence seen in *Ceratodon purpureus* helps explain its restriction to drier sites and conversely, the low tolerance of desiccation and high tolerance of submergence displayed by the endemic *Grimmia antarctici* is consistent with its restriction to wet habitats. Finally the flexible response observed for *Bryum pseudotriquetrum* is consistent with its co-occurrence with the other two species across the bryophyte habitat spectrum. The likely effects of future climate change induced shifts in water availability are discussed with respect to future community dynamics.

Introduction

The Antarctic terrestrial environment is largely devoid of herbivores and many other biological factors, such as disease, which normally influence vegetation dynamics are reduced or absent in this cold-dominated environment. Plant community dynamics are therefore primarily shaped by abiotic environmental constraints (e.g. low temperatures and water availability) and/or interspecific competition. An understanding of plant responses to abiotic variables is therefore particularly important for understanding plant community dynamics in this environment. By investigating plant-responsiveness to abiotic variables we aim to understand how niche partitioning in the suite of study species might shape future communities under predicted climate change. We aim to identify environmental shifts that might cause species exclusion and/or improved competitive advantage.

Under a climatic warming scenario it is arguably the indirect changes in key environmental growth variables, such as water availability, that will be of greater influence to the growth environment than that of temperature alone (Chapin 1983, Hobbie and Chapin 1998). Whilst the details of how climate change is likely to affect water availability specifically (e.g. degree, length and frequency of dry periods) are yet largely unknown, water availability is likely to increase in the short term, because of increased summer melt in response to warming conditions. However, in the long term increasing aridity is likely, if predicted increases in precipitation are not adequate to replenish the permanent snow and ice reserves which provide summer water (Tokioka 1995, Ye and Mather 1997; Robinson *et al.* 2003). Persistence under future climates may depend on a species ability to withstand physiological stress events associated with these changes to water availability, namely desiccation and submergence.

In the terrestrial Antarctic environment, bryophyte communities are restricted to relatively wet habitats that receive free water during the summer melt. Desiccation and submergence events commonly occur during the summer growth season, with submergence restricted to melt periods. Within the present study area, the Windmill Islands, East Antarctica, the flora is entirely cryptogamic (consisting of approximately 27 lichen taxa, three bryophyte species, a single liverwort and a suite of terrestrial alga and cyanobacteria). The focus of the present study are the three bryophytes (*Grimmia antarctici* Card. ≈ *Schistidium antarctici* Card., *Bryum pseudotriquetrum* (Hedw.) Gaertn., Meyer & Scherb. and *Ceratodon purpureus* (Hedw.) Brid.) whose distributions are determined by water availability. *Grimmia antarctici* is restricted to the wettest sites, *C. purpureus* to the driest sites and *B. pseudotriquetrum* co-occurs with both (Selkirk and Seppelt 1987, Wasley 2004). From these patterns and also field observations (J. Wasley *pers. comm.*), *G. antarctici* is the species most commonly subject to submergence and *C. purpureus* is the species most commonly subject to desiccation. Whilst it has been previously demonstrated that *C. purpureus* has a relatively high tolerance of desiccation (Robinson *et al.* 2000) tolerance of submergence has not been reported for any of these mosses. By comparing tolerance of both desiccation and submergence we aim to assess the role water availability plays in driving species distributions. Specifically, to determine whether *G. antarctici* is absent from dry sites and *C. purpureus* absent from wet sites due to intolerance of desiccation and submergence respectively.

The ability to avoid desiccation is limited in bryophytes. Bryophytes are non-vascular and their gametophytes therefore do not possess many of the mechanisms for desiccation avoidance that characterise vascular plants. Within bryophyte turf, water that is stored both internally and externally plays a functional role. Most water

conduction in these plants occurs externally, but metabolism of individual cells relies on internal water content only (see Proctor 2000 for review). Some aspects of bryophyte turf structure can however alter a species' ability to retain moisture. A tightly packed turf, with few inter-shoot spaces will be less prone to drying than a loosely packed turf that has large spaces between shoots. Shoot size and density were therefore determined for turf samples of each of the three Windmill Island moss species.

The ability to tolerate drying is a characteristic that enhances a species' tolerance of desiccation, and ultimately survival under a drying climate. The ability to metabolise at relatively low turf water content (TWC) and thus photosynthesise during desiccation, will allow a plant to perform better under drying conditions. TWC at which photosynthetic efficiency declines was therefore determined, for each of the three species, over the course of a summer season.

The ability to survive desiccation is often due to the presence of compounds which protect membrane structure during the desiccation / rehydration process. Such compounds include particular types of soluble carbohydrates and lipids. In addition to providing energy rich storage, fatty acids are important for cell membrane structure and function (Hulbert 2003), and fatty acid composition is important in development of chilling and freezing tolerance. Various soluble carbohydrates can provide protection during desiccation and freezing (Roser *et al.* 1992, Montiel 2000, Robinson *et al.* 2000) and bryophytes are known to contain soluble carbohydrate contents that parallel those in the embryos of maturing seeds (Proctor 2000). Since the quantity and type of soluble carbohydrate and lipid compounds can be important factors for protection during desiccation in plants (Koster and Leopold 1988, Ghasempour *et al.*

1998), concentrations of these biochemical factors were determined for the study species.

The ability to recover from desiccation will contribute toward a species' tolerance of desiccation. Species that are able to recover from complete desiccation of tissues will have a greater chance of surviving a drying climate. Further, species that are able to recovery quickly from desiccation will have greater productivity under a drying climate where plants will need to take full advantage of short periods of water availability. Recovery time, after complete desiccation of plant material, was therefore measured for the study species, using chlorophyll fluorescence.

Since plants occupying relatively wet habitats are less likely than those that occupy dry environments to survive a drying trend, information about water availability in the current and past growth environment is also important. Carbon isotopic fractionation can offer insight into water availability in the growth environment (Rice and Giles 1996). Specifically, fractionation of carbon isotopes provides information on access of CO_2 to the photosynthetic enzyme Rubisco. More positive $\delta^{13}C$ values are indicative of less photosynthetic isotopic fractionation, reflecting either that plants have some sort of carbon concentrating mechanism (as found in algae, C4 or Crassulacean acid metabolism plants), or more likely that there are diffusional limitations to CO_2 reaching the site of Rubisco. In higher plants this diffusional fractionation relates to stomatal opening. However in cryptogams, which lack stomata, it likely reflects the degree to which plants are submerged in water and the consequent diffusional limitations (Proctor *et al.* 1992, Bottger *et al.* 1993, Rice and Giles 1996). Cryptogams subject to submergence are expected to show elevated (less negative) $\delta^{13}C$ signatures. Carbon isotopic fractionation was therefore measured for each species as an indication of degree of habitat submergence and, for *G. antarctici*,

changes in fractionation with depth (and age) of gametophytes were examined to determine historic changes in habitat submergence.

The overall aims of the study were three-fold. Firstly, to compare the relative tolerance of submergence and desiccation of the three study species and secondly, to determine what tolerance of desiccation strategies are employed by the three species? Specifically what is their relative ability and/or what traits do they exhibit to: (a) avoid, (b) tolerate, (c) recover from and (d) facilitate membrane function during desiccation? And finally, with respect to submergence, is there variation in degree of submergence of present habitats and over past environments.

Based on present species distributions, we hypothesise that (1) *G. antarctici* will show relatively high tolerance of submergence and relatively low tolerance of desiccation (2) *C. purpureus* will show relatively high tolerance of desiccation and relatively poor tolerance of submergence (3) *B. pseudotriquetrum* will have an intermediate response to both desiccation and submergence.

Methods

Plant material

Sample material of the three study species (*B. pseudotriquetrum*, *C. purpureus* and *G. antarctici*) was collected from Antarctic Specially Protected Area (ASPA) 135, on Bailey Peninsula, approximately 1 km east of Casey Station. The collection site was a low-lying soak area close to the northern boundary of ASPA 135, at the top of a snow slope above Thala valley (at 66˚16.92'S, 110˚32.36'E). All three species were collected from within an area of approximately 4 m^2 where they grow in a largely continuous carpet, inter-dispersed with fellfield rocks and boulders. Within the site the

three study species occupy a water gradient, as described above, with *G. antarctici* the dominant species.

Material was collected at three times over the Southern Hemisphere summer season of 1999-2000: early-season (2/12/99), mid-season (24/1/00) and late-season (27/2/00). The early- and late-season collections were before and after the summer-melt period, respectively, and as a consequence the plant material was frozen and relatively dry. In contrast, the mid-season collection coincided with the height of the summer melt, during which the material was relatively warm and wet, allowing an investigation of bryophyte response to seasonal changes in water availability. Morphological measurements, fatty acid determination and isotopic analysis were performed on only one set of samples over the season (late-season), whilst desiccation tolerance and carbohydrate analysis, were measured at each of the three times.

Tolerance of desiccation

At each collection time, a turf sample of approximately 50 mm x 30 mm was collected for each of the three species and transported immediately to the laboratory. In the laboratory, the turf samples were sprayed with water and allowed to hydrate overnight. Replicate plugs of moss (approximately 13 mm diameter and 10 mm depth) for each species were cut from the rehydrated material (n = 8 for early- and late-season, 6 mid-season). Excess surface water was removed by blotting samples with paper towel. The samples (approximately 1.5 g fresh weight) were placed in small pre-weighed foil capsules.

Chlorophyll fluorescence was used to assess the ability to photosynthesise during desiccation and the recovery of photosynthesis following desiccation for the three species throughout the season using methods adapted from Robinson *et al.* (2000).

Maximum quantum yield of PSII (F_v/F_m) was measured for each sample after a 10 min dark adaption period, using a Mini PAM chlorophyll fluorometer (H. Walz, Germany). Moss samples were then allowed to desiccate slowly in the laboratory under low light conditions (approximately 10 μmol photons m^{-2} s^{-1}), air temperature 18°C, and relative humidity 22%. F_v/F_m and sample weights were determined periodically for each sample until F_v/F_m declined to zero and/or minimal fluorescence (F_o) values were too low to consider reliable. The samples were then stored at low light until the recovery phase of the experiment was conducted.

Change in the turf water content (TWC) of each sample was determined from changes in weight measured immediately after each measurement of chlorophyll fluorescence, as described in Robinson et al (2000). In the context of this experiment, the external water stored between gametophyte shoots is biologically important to the physiological functioning of the turf as a whole (see also Wardlaw 2005 for general discussion of importance of apoplastic water). A species that is better able to retain external water will have a slower drying rate and therefore be able to delay desiccation compared to a species that has little external water and a fast drying rate.

Recovery from desiccation

Recovery from desiccation was measured approximately 1 week after desiccation commenced. Replication was half that of the desiccation phase of the experiment as half the samples were used in biochemical analyses described below (n = 4 for early- and late-season, 3 mid-season). The desiccated moss samples were dark adapted for 10 min, sprayed with a fine mist of water to commence rehydration and F_v/F_m measurements started immediately. Recovery from desiccation was conducted in the dark for approximately 1 h and in low light conditions thereafter until F_v/F_m values stablised. During recovery plant moisture was maintained with regular spraying.

Since recovery of F_v/F_m was too fast to measure TWC simultaneously, recovery was therefore measured over time alone.

Tolerance of submergence

Tolerance of submergence was measured for naturally submerged *G. antarctici* and *B. pseudotriquetrum* and under experimentally manipulated submerged conditions for all three study species. The experimentally manipulated submerged conditions were created using moss turf of the three species collected on 19/12/02 from the above described ASPA 135 site, from which six replicate samples of each species (1 cm^2 area, 1.5 cm deep) were prepared. Replicates were inserted into 12 mm diameter holes in sponges (Scotch-Brite Multi-Purpose, Thick, 3M, USA) that were placed in perspex petri dishes (8.5 cm diameter) and attached to lengths of flat aluminium bar (80 cm x 3 cm) with wire. Samples were secured by a square of fine wire mesh (5mm grid) over the top of each petri dish.

Effective quantum yield of PSII (ΦPSII) was measured using chlorophyll fluorescence. Initial ΦPSII was measured prior to submergence of the material. The samples were then submerged in a melt lake, located behind the Casey Station accommodation building on Bailey Peninsula (66˚15.9'S, 110˚31.6'E), at a depth of approx 5 cm. ΦPSII was monitored approximately weekly over a 6 week period (19/12/02 to 30/1/03). ΦPSII was also measured ten times at three sites on three naturally occurring, submerged samples of *B. pseudotriquetrum* and *G. antarctici*. *Ceratodon purpureus* was not included in this set of measurements as no naturally occurring submerged material could be found.

Habitat submergence ($\delta^{13}C$)

Species differences in $\delta^{13}C$ were determined from samples collected in association with the final season desiccation experiment. Longer-term changes (decadal) of habitat water availability were investigated by measuring $\delta^{13}C$ along long gametophytes of *G. antarctici* (collected from the edge of the melt lake used for submergence experiments above). The moss turf here contained continuous intact gametophytes up to 4 cm long. Five turf cores were sectioned at 1 cm intervals and isotopic determinations performed on each individual section. The short gametophyte length of the other species prevented this experiment being performed for *C. purpureus* and *B. pseudotriquetrum.* Plant material was oven dried at 70 °C and ground to a fine powder (0.2 mm particles) in a ball mill (Retsch MM2). Dried, ground material was analysed using mass spectrometry methods, after Hietz *et al.* (1999) as described in Wasley *et al.* 2006 (in press).

Morphology

The growth form of these Antarctic bryophytes is a compact turf comprised of entire gametophyte shoots that may extend for several cm, the top few mm at the tip being green and photosynthetic. In contrast to traditional peat there is little soil or organic debris under the turf. These bryophyte turfs hold water intracellularly (within gametophytes) and extracellularly (in inter-shoot spaces). Inter-shoot space is a factor of (1) gametophyte shoot density and (2) gametophyte shoot width. These morphological characteristics were determined for naturally desiccated samples collected in association with the late-season desiccation experiment.

Gametophyte shoot density was determined using a dissecting microscope. Quadrats (2 x 2 mm) were placed in three random locations on each replicate moss plug and the

number of gametophytes within each quadrat was counted. The median value of the three counts was used for analysis. Samples were then air-dried in a desiccator, at –4 ˚C, prior to measurement of shoot widths. Eight single gametophyte shoots, for each of the three species, were randomly selected, with juvenile and/or dead shoots excluded from the selection. Measurements were conducted using binocular microscopes (Leica MS5 and Leica Wild M3C) with a graticule. Shoots were measured at the widest part of their tips. Measurements were repeated three times for each shoot and the median value was used for analysis.

Fatty acids

Fatty acid composition was determined for three replicate samples of each species, collected in association with the late-season desiccation experiment. Extraction and methylation of the samples followed Liu *et al.* (2000). Identification and quantification of fatty acids was conducted using gas chromatography-mass spectrometry, also as described in Liu *et al.* (2000).

Soluble carbohydrates

Samples for soluble carbohydrate analysis were collected in association with each of the three desiccation experiments described above (n = 8 for early- and late-season, 6 mid-season). Samples were hydrated overnight, microwaved on high power for 2 min to preserve organic compounds (Popp *et al.* 1996) and dried and ground as described for $\delta^{13}C$. Hot water extracts (40 mg sample in 1 mL dd H_20) were prepared and de-ionised on ion exchange resins. Low molecular weight carbohydrates and sugar alcohols in the neutral fraction were derivatised and analysed as their trimethylsilyl ethers using gas chromatography (Hewlett Packard HP 6890 Gas Chromatograph) as described in Richter *et al.* (1990). Calibrations were performed with a wide range of

available standards. For unidentified peaks the calibration curve of the standard with the closest retention time was used. Retention times for unidentified peaks (X2, X3 and X4) were 27.98, 14.14 and 15.12 min, respectively. X2 eluted close to raffinose and is likely a relatively large compound, whilst X3 and X4 are closer in size to the hexoses (glucose and fructose).

Statistical analysis

Desiccation and recovery from desiccation was analysed using non-linear regression. Each replicate was analysed by plotting (1) F_v/F_m against TWC during desiccation and (2) F_v/F_m against time during recovery. During the desiccation phase of the experiment F_v/F_m typically remained high (≈ 0.8) until the water content of the plant material declined to a critical point (herein referred to as the critical water content; CWC; g H_2O^{-1} g dw), after which F_v/F_m sharply declined toward zero. The recovery phase was typified by the reverse trend, with F_v/F_m increasing sharply following hydration until a critical point (herein referred to as the critical recovery time; CRT; min), after which F_v/F_m plateaued. Non-linear regression analysis was performed for each plot in order to determine the intercept points, CWC and CRT, which were further analysed as described below.

Statistically significant differences within each of the following data sets were tested using ANOVA: CWC; CRT; tolerance of submergence; $\delta^{13}C$; turf morphology; fatty acids and soluble carbohydrates. Where required, the data sets were transformed to normalise the variance of the data and thereby satisfy the assumptions of the ANOVA model. Pairwise comparisons (using Student's t test) were used to identify pairs of means which are significantly different. All statistical operations were conducted using JMP (SAS Inc., Cary, NC, USA) statistical analysis software.

Results

Tolerance of desiccation

Critical water content ranged between approximately 0.5 to 2 g H_2O g^{-1} dw (Fig. 1). Significant variation was observed across the three species, and three experimental intervals (whole model and time*species interaction, $P<0.0001$; Table 1). CWC was highest in *B. pseudotriquetrum* early in the season (December), when mean CWC approached 2 g H_2O^{-1}g dw, but declined by half over the course of the season (Fig. 1). *Grimmia antarctici* CWC was consistently high throughout the season whilst *C. purpureus* CWC was consistently lowest (Fig. 1). By the end of the season, CWCs of *B. pseudotriquetrum* and *C. purpureus* had reached similar low levels and were significantly lower than those for *G. antarctici* ($P<0.003$).

All three species were able to survive desiccation and recover photosynthetic activity within an hour of rehydration. In *B. pseudotriquetrum* and *C. purpureus*, CRT increased over the course of the season ($P<0.003$; Fig. 1), while *G. antarctici*, showed a significant CRT seasonal decline ($P<0.003$; Fig. 1).

Tolerance of submergence

Grimmia antarctici and *B. pseudotriquetrum* performed similarly under submerged conditions, in both naturally occurring and experimentally created environments. Naturally submerged bryophyte turf had ΦPSII values of 0.57±0.01 for *B. pseudotriquetrum* and 0.59±0.01 for *G. antarctici* (mean±se, n = 30). Under experimental simulations, *C. purpureus*, the study species absent in naturally occurring submerged habitats, had significantly lower ΦPSII than the other two study species ($P<0.0001$; Table 1), with ΦPSII declining to 15% of start values by 14 days

(Fig. 2). ΦPSII remained above 50 and 60% of start values for *B. pseudotriquetrum* and *G. antarctici*, respectively, for the entire 6-week experimental period (Fig. 2).

Habitat submergence and morphology

Values of $\delta^{13}C$ (mean±SEM, n=8) were significantly less negative in *G. antarctici* (-22.87±0.10) than in either *C. purpureus* (-25.47±0.11) or *B. pseudotriquetrum* (-25.48±0.08), suggesting that the former species is exposed to greater diffusional limitations (P <0.0001; Table 2). In long *G. antarctici* gametophytes, $\delta^{13}C$ increased significantly from the growing tip to the oldest and deepest tissue (P = 0.0004; Table 2 and Fig. 3), suggesting that the environment has been drier in the recent past.

Shoot widths (mean±SEM, n=8) were significantly different between the three species (P<0.0001; Table 2) with approximately 0.1 mm difference between each species; *B. pseudotriquetrum* was the largest (0.51±0.03), *C. purpureus* intermediate (0.41±0.02) and *G. antarctici* the smallest (0.31±0.01). Desiccated turf shoot density was greatest in *C. purpureus* (888±47.5 shoots cm^{-2}, P<0.0001; Table 2) whilst *B. pseudotriquetrum* and *G. antarctici* showed similar shoot densities (550±70 cm^{-2} and 550±52.5 cm^{-2}, respectively). A schematic illustrating the relative size and packing of the three species within a turf is shown in Fig 4. Because of its small size and low density, *G. antarctici* has greater inter-shoot spaces and therefore a more loosely packed turf than the other two species.

Fatty acids

Fatty acids are important for membrane function and they may also provide protection during desiccation and freezing in these Antarctic mosses. A total of 14 fatty acid compounds were detected at concentrations of >0.1 $mg^{-1}g$ dw (Appendix 1). *Ceratodon purpureus* was fatty acid rich, containing 2- and 3-fold greater total fatty

acid concentrations than *B. pseudotriquetrum* and *G. antarctici*, respectively (P = 0.0015; Table 2 and Fig. 5). The lipid composition of all three species was predominantly polyunsaturated fatty acids (PUFA; 70 to 85% of total), saturated fatty acids (SFA) constituted up to 30% of total fatty acid composition and monounsaturated fatty acids (MUFA) were a minor component, occurring at concentrations of up to 5% of total (Fig. 5). The ratio of unsaturated fatty acid (UFA) to SFA was greatest in *C. purpureus* (3.77±0.24), intermediate in *B. pseudotriquetrum* (3.21±0.49) and lowest in *G. antarctici* (2.82±0.13). The most abundant fatty acids in all species were the SFA 10:00, and the PUFAs 18:2 and 18:3, which occurred in relatively high concentrations (> 1 mg^{-1}g dw) in all three species (Appendix 1). Other relatively abundant fatty acids included SFAs 16:0 and 18:0, the MUFA 18:1 and a suite of PUFAs (18:2, 18:3 n-6, 20:2, 20:3 n-6, 20:4, 20:3 n-3, 20:5 and 22:5; Appendix 1).

Soluble carbohydrates

Eight identified and three unidentified soluble carbohydrates were detected across the season (Appendix 2). This suite of soluble carbohydrates is similar to that previously reported for these species (Robinson *et al.* 2000). However, of the five unidentified soluble carbohydrates previously reported only X2, X3 and X4 were found in the current study.

Soluble carbohydrate concentrations were highest ($\geq$200 mg g^{-1} dw) early in the season in both *B. pseudotriquetrum* and *G. antarctici*. In these two species, total soluble carbohydrate concentrations showed a significant decline over the season, but remained above 90 mg^{-1} g dw throughout (Appendix 2). *Bryum pseudotriquetrum* showed the highest soluble carbohydrate concentrations ($\geq$270 mg g^{-1} dw) but also exhibited the greatest seasonal decline over the season (>50% Appendix 2).

Ceratodon purpureus contained low soluble carbohydrate concentrations throughout the season (total < 70 mg^{-1} g dw) and showed no significant seasonal fluctuation. Total soluble carbohydrate concentrations, for the late season experiment, were two-fold greater in *G. antarctici* than *C. purpureus* (P<0.0001; Table 1 and Fig. 5).

Several of the soluble carbohydrates detected are known to play a role in tolerance of desiccation, particularly stachyose, trehalose and the sugar alcohols (glycerol, mannitol and *myo*-inositol). *Bryum pseudotriquetrum* was the only species to contain stachyose and contained significantly higher concentrations of trehalose than the other two species (P<0.05; Fig. 5). The three sugar alcohols (glycerol, mannitol and *myo*-inositol) were also found in all species, but at low concentrations (<2 mg^{-1} g dw).

Discussion

Biological profiles underpinning tolerance of desiccation

Biological traits that underpin a species' relative chance of surviving a drying climate were investigated through the development of biological profiles that relate to tolerance of desiccation. Increased tolerance of drying conditions was considered as the ability to (1) avoid desiccation, (2) tolerate drying, (3) recover from desiccation, and (4) produce compounds facilitating membrane function during desiccation events.

Avoidance of desiccation

Turf morphology measures show that *G. antarctici* has a relatively loosely packed turf (Fig. 4), indicating that this species has reduced desiccation avoidance potential. The other two species had more densely packed turf, and therefore potentially greater desiccation avoidance. Other morphological characteristics that influence desiccation avoidance, such as degree of leaf curling toward stem (Longton 1988), might also be adopted by these species and are worthy of further study.

Despite differences in shoot packing between *B. pseudotriquetrum* and *G. antarctici*, these species have similar TWC at full hydration (Wasley 2004). *Bryum pseudotriquetrum* therefore stores a greater proportion of its turf water internally than the loosely packed turf of *G. antarctici*, which exhibits relatively high external water storage. Since external water is lost more quickly than internal water, this provides further support for *G. antarctici* having the lowest desiccation avoidance potential.

Turf densities in the present study were exceptionally high, with *C. purpureus* approaching 900 shoots per cm^2, a density which is 75% greater than that measured in European populations of this species (Simon 1987).

Tolerance of desiccation

Tolerance of desiccation was quantified as the ability for a species to continue to metabolise at low water contents. Water content in this context was inclusive of both internal and, in this group of plants, the intimately linked external water. Distinguishing between these two sources was beyond the scope of this study, but warrants future work.

Highest tolerance of desiccation was found in *C. purpureus*, in which photosynthetic activity was maintained at relatively low water contents throughout the season (Fig. 1). Conversely, in *G. antarctici* photosynthesis required relatively high water contents (ceasing below ~1.2 g H_2O^{-1}g dw; Fig. 1), suggesting that this species is relatively intolerant of desiccation. A third response pattern was observed in *B. pseudotriquetrum*, which showed considerable flexibility in its response to desiccation, and displayed a significant seasonal decline in CWC (Fig. 1). We have previously shown that plants of this species collected from dry sites maintained metabolism to lower CWC than plants from wet sites (Robinson *et al.* 2000),

suggesting the possibility of ecotypes. However, the present study clarifies that plants from the same site demonstrate a seasonal response to desiccation showing that acclimation occurs.

Recovery from desiccation

Recovery from desiccation was quantified by measuring critical recovery time (CRT), the time from rehydration to the point at which photosynthetic activity had largely recovered and the rate of increase had begun to plateau. CRT slowed in *B. pseudotriquetrum* and *C. purpureus* across the season (Fig. 1). This reduction in overall tolerance to desiccation throughout the season could represent an acclimation response related to temperature and not simply water availability. Since *C. purpureus* occupies the most xeric habitats (Selkirk and Seppelt 1987; Wasley 2004), which are likely to be the first to dry, this species may show winter acclimation earlier than the other species. *Grimmia antarctici* was the only species to show a seasonal decline in CRT, and an overall increase in tolerance of desiccation across the season, with the fastest recovery of all in the late-season experiment (CRT <5 min; Fig. 1). These recovery results contrast with a maritime Antarctic study where recovery was slower in hydric than xeric moss species (Davey 1997).

It should be noted that recovery from desiccation occurred within an hour, in all species and times. This is much faster than that reported in a similar fluorescence study of the resurrection angiosperm, *Chamaegigas intrepidus* (Woitke et al 2004). The advantage gained by a quicker CRT is thus in the order of minutes. In contrast, the ability to metabolise at low TWC is likely to allow photosynthetic carbon gain to occur over periods of hours. As such, CWC is probably a more important indicator of overall tolerance of desiccation in these species than CRT.

Biochemical factors which could aid desiccation tolerance

An important characteristic that assists in survival of desiccation is the ability to regain membrane function, quickly and efficiently, after a desiccation event. Many compounds are important in promoting membrane integrity, during desiccation and rehydration, including a suite of soluble carbohydrates and fatty acids.

Bryum pseudotriquetrum contains a high proportion of soluble carbohydrates which are known to play an important role in desiccation and freezing tolerance. For example it was the only species in which stachyose was detected and this sugar was a substantial component of the overall pool (Fig. 5). Stachyose is known to play a role in desiccation tolerance and is commonly found in other desiccation tolerant organisms such as invertebrates and vascular plant seeds (Koster and Leopold 1988, Pukacka and Pukacki 1997). Whilst stachyose was absent from the other two species, it has been previously detected in these species, albeit in trace concentrations (Robinson *et al.* 2000). This species also contained the highest concentration of trehalose which may be important in promoting desiccation tolerance, as discussed below. The unidentified X4, also found in *B. pseudotriquetrum* (Appendix 2), is a small soluble carbohydrate similar in size to glucose and fructose and its function is unknown at this stage. However, the high concentration of X4 early in the season would also contribute toward the total soluble carbohydrate concentrations in this species, and therefore is likely to promote overall osmotic stabilisation of tissues, counteracting damage during desiccation.

Trehalose, a cryoprotective carbohydrate commonly found in polar biota (Montiel 1998, 2000, Weinstein *et al.* 2000), was detected in all three species, despite not being detected in these species in a previous study from the same region (Melick and Seppelt 1992). This compound is common in many organisms capable of

withstanding near-complete desiccation, including cyanobacteria, fungi, yeast, arthropods and plant seeds, as it is very effective at stabilizing proteins and other macromolecules during dehydration (Crowe *et al.* 1992). It has been suggested that caution should be exercised, however, regarding the detection of trehalose in field-collected, continental Antarctic bryophytes; as it usually occurs only in low levels, and is absent from laboratory grown *C. purpureus* and temperate moss species (Roser *et al.* 1992). Roser *et al.* 1992 suggest trehalose maybe derived from epiphytic fungi rather than bryophyte tissue, however if this is the case it would indicate high fungal contamination.

The high levels of soluble carbohydrates that occurred early in the season in both *B. pseudotriquetrum* and *G. antarctici* (Appendix 2) probably reflects active photosynthesis early in the season, as photosynthetic activity occurs while plants are still snow covered (Collins and Callaghan 1980). The highest soluble carbohydrate concentrations occurred in *B. pseudotriquetrum*, as did the largest seasonal decline (Fig. 5.6). This is in contrast to other Antarctic studies, from both the maritime (Davey 1999) and continental (Melick and Seppelt 1994b) regions, which found no significant seasonal change in carbohydrate contents, or found concentrations to increase during the summer (Zuniga *et al.* 1996). Despite Melick and Seppelt (1994b) reporting no significant annual cycle in soluble carbohydrate contents for *B. pseudotriquetrum*, or *G. antarctici*, concentrations in these species, collected from the same peninsula a decade earlier, showed similar concentrations and a similar decline to that found in the present study over the same collection period (December to February). The seasonal decline in soluble carbohydrates in both species may reflect a lag between the start of photosynthesis early in the season and the onset of growth, however *B. pseudotriquetrum* has also been reported to lose a greater portion of its

sugar pool during freeze-thaw events over the summer season, relative to the other study species (Melick and Seppelt 1992).

The lowest soluble carbohydrate concentrations were found in *C. purpureus* (Fig. 5), but concentrations for this species were still within the range found for the three study species in a previous study (Roser *et al.* 1992) and in arctic *Polytrichum* (Sveinbjornsson and Oechel 1991, Barsig *et al.* 1998). Conversely, *C. purpureus* had the highest concentration of fatty acids and the ratio of total fatty acids: identified soluble carbohydrates was approximately 5-fold higher in *C. purpureus* (0.756) than the other two species: *B. pseudotriquetrum* (0.158) and *G. antarctici* (0.145). Since it is unlikely that this species has more membrane material, the elevated fatty acid concentrations likely indicate greater use of fatty acids for storage. *Ceratodon purpureus* appears to be converting a greater proportion of its carbon pool into fatty acids, while the other two species retain significantly more in the form of soluble carbohydrates. A similar increase in the relative concentration of fatty acids to carbohydrates is seen in evergreen trees growing at altitude compared with lowland trees (Li *et al.* 2002) and in herbaceous biomass along an altitudinal gradient on Mt Olympus, Greece (Pantis *et al.* 1987). Lipids are suggested to form an efficient, osmotically neutral form of carbohydrate storage in these alpine plants. It seems likely that *C. purpureus* relies more on its large lipid pool, than its sugar pool, for protection from freezing and desiccation. Carbon storage in this energy rich, but insoluble form may also reduce the loss of soluble carbon during desiccation and freeze-thaw events. Temperate *C. purpureus* has also been shown to contain a relatively high concentration of lipids, often contained in cytoplasmic droplets (Swanson *et al.* 1976). Nutrient stressed, axenic cultures of *C. purpureus* showed an almost 4 fold increase in such triglycerides especially 9,12,15–Octadecatrien–6-ynoic acid (Andersson *et al.*

1974). Fatty acids with 4 and 5 double bonds (such as arachidonic and eicosapentaenoics) are uncommon in higher plants (Gellerman *et al.* 1972) but not unusual in mosses (Anderson *et al.* 1974).

Along with a higher concentration of total lipids, *C. purpureus* contains a different suite of lipids to the other two species, with more PUFAs and less SFA. This species had the highest UFA:SFA ratio, within the upper range typically found for chilling-resistant plants (Lyons *et al*. 1964). Whilst *G. antarctici* was found to have a low UFA:SFA ratio, within the range considered to be typical of chilling-sensitive plants. The high proportion of UFAs found for *C. purpureus* indicates that this species may have different membrane composition to the other species, and this may in turn be advantageous in allowing it to compete better in an environment with frequent freeze-thaw or desiccating events. Since photosynthesis and respiration are both membrane based processes it is possible that this unusual lipid composition explains the ability of this species to maintain photosynthesis under the driest conditions.

Tolerance of submergence and habitat submergence

The distribution of the three species along a water gradient (Selkirk and Seppelt 1987 and Wasley 2004) indicates that they are likely to have different biological requirements of the growth environment and occupy separate ecological niches. Since climate change is expected to cause shifts in water availability in this environment the present water-relations of these species offer clues as to their ability to survive future altered conditions (Robinson *et al*. 2003).

The field manipulated submergence experiment presented in this study is the first of its kind to be conducted on the Antarctic continent. Submergence is not typically considered in relation to continental Antarctic vegetation because, with most water

locked away as snow and ice, the Antarctic continent is largely a desert environment – hence tolerance of desiccation has received limited research attention. The present study demonstrates, however, that submergence events may play a role in shaping these vegetation communities. *Ceratodon purpureus*, for example, showed significantly lower photosynthetic efficiency under submerged conditions, providing an explanation for its exclusion from wet habitats in the Windmill Islands.

The $\delta^{13}C$ signature for the three species concurs with the trends presented in Selkirk and Seppelt (1987) and Wasley (2004), suggesting that the two cosmopolitan species occupy drier sites and *G. antarctici* is dominant in submerged habitats, such as lake edges and ephemeral pools that appear during the peak of the summer melt. *G. antarctici* is thus subject to greater diffusional limitations. The habitat occupied by this latter endemic, species is therefore less likely to persist under a drying climate than the conditions presently occupied by the other species. The changes in $\delta^{13}C$ with depth of gametophyte support the current drying trend, described by Melick and Seppelt (1997) and suggest that submergence was more common in the past than it is at the present time (Fig 3). The gametophytes used were entire with the lower sections representing growth that occurred in previous years. Given this structural integrity. it is unlikely that decomposition of tissues is a major contributor to this isotopic fractionationation and the lack of transport tissues mean that mixing between layers is also unlikely. Based on estimates of growth rates of 0.6 mm per year for this species in the Windmill Island (Melick and Seppelt 1997) these gametophytes are likely to be over 60 years old, predating the availability of meteorological data for the region.

Conclusions

Our results indicate that the three moss species occupy distinctly different ecological niches with respect to water relations. The trait differences between the three species with respect to their response to the two stress events – desiccation and submergence – are summarised in Table 3.

The key desiccation and submergence characteristics found for *B. pseudotriquetrum* include its flexible tolerance of desiccation. This species showed high desiccation avoidance potential via its relatively tightly packed turf. Its ability to survive desiccation is likely enhanced by its stachyose content, a soluble carbohydrate known for its role in tolerance of desiccation. In addition to properties that are likely to facilitate tolerance of desiccation, *B. pseudotriquetrum* also performed well at the other physiological extreme by showing high tolerance of submergence, which further supports the notion of physiological flexibility in this species.

Conversely, *C. purpureus* was characterised by a high tolerance of desiccation but low tolerance of submergence. In addition, this species has a high ability to avoid desiccation, as its tightly packed turf is expected to promote moisture retention. Membrane function during desiccation events is likely to be facilitated by its high polyunsaturated fatty acid content.

Finally, *G. antarctici* showed an opposite response to *C. purpureus*. This species showed a low tolerance of desiccation, as it required high water contents throughout the season to maintain metabolism. Compounding poor tolerance of desiccation, turf morphology suggests this species is most prone to drying as it has a relatively loosely packed turf. In contrast, this species showed highest tolerance of submergence and its $\delta^{13}C$ signatures suggest that this species is subject to relatively frequent submergence.

These patterns in physiological tolerance of desiccation and submergence for the three species provide an explanation for current species distributions. The poor tolerance of submergence seen in *C. purpureus* helps explain its restriction to drier sites and conversely, the low tolerance of desiccation and high tolerance of submergence displayed by *G. antarctici* is consistent with its restriction to wet habitats. Finally, the flexible response observed for *B. pseudotriquetrum* is consistent with its co-occurrence with the other two species across the bryophyte habitat spectrum.

Antarctic terrestrial bryophyte communities are restricted to low-lying wet areas that receive adequate free-water during the growth season. Under a drier climate bryophyte habitat is likely to contract. Previous studies have shown that *G. antarctici* is more at risk from UV-B radiation damage (Lovelock and Robinson, 2002, Robinson et al 2005). The results of the present study suggest that habitat drying and subsequent contraction of species' distributions also places this Antarctic endemic at risk of extinction.

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Figures

Figure 1: Desiccation and recovery responses across a summer season (December, January and February) for *Bryum pseudotriquetrum*, *Grimmia antarctici* and *Ceratodon purpureus*. The relationship between the turf water content at which F_v/F_m began to decline during desiccation (critical water content; CWC; g H_2O g^{-1} dw) and the time taken for the fluorescence signal to recover (after rehydration (critical recovery time: CRT; min) is shown for early (filled circles), mid (open circles) and late (filled triangles) season experiments.

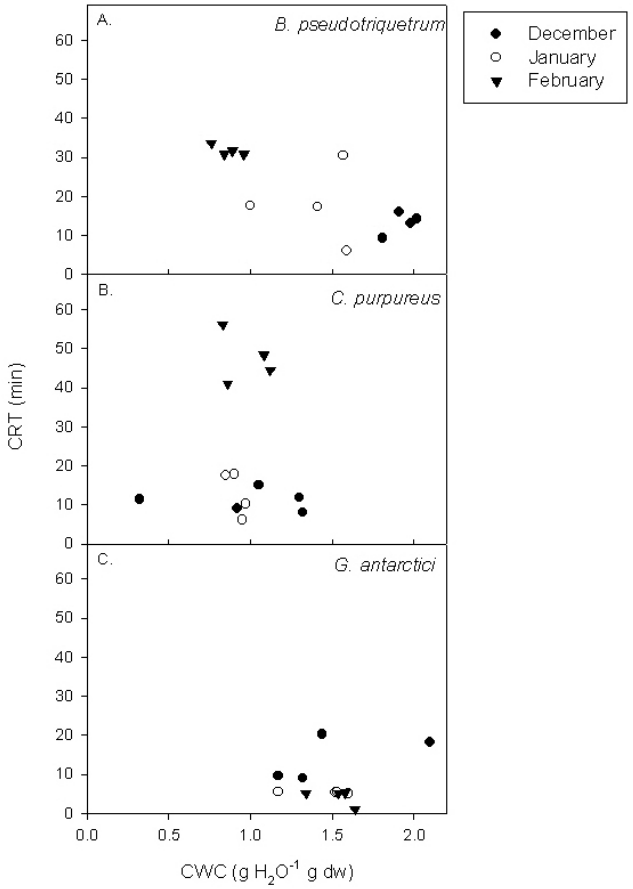


Figure 2: Effective quantum yield for *Bryum pseudotriquetrum*, *Grimmia antarctici* and *Ceratodon purpureus* subject to 42 days submergence in the field (A, Mean±sem, n = 6. Photon Flux Density (PFD) at the time of ΦPSII measurements (B, mean±sem, n = 18).

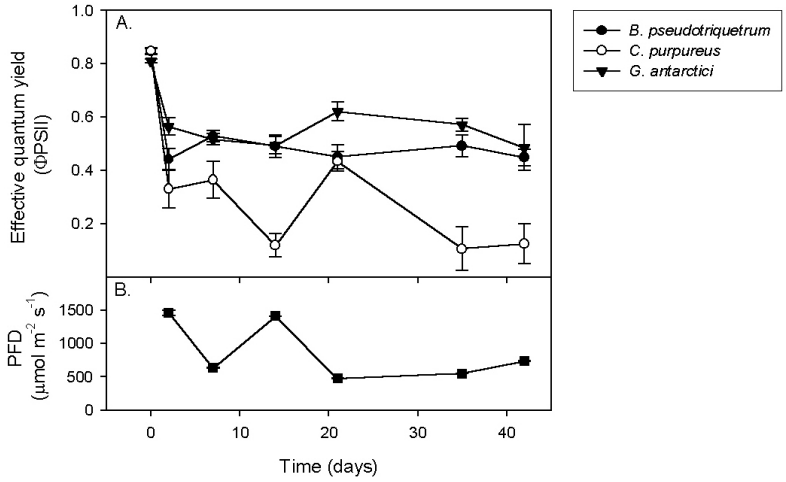


Figure 3: Changes in $\delta^{13}C$ signature with depth for 4 cm long gametophytes of *Grimmia antarctici* collected from Bailey Peninsula (Data are mean±sem, n = 5).

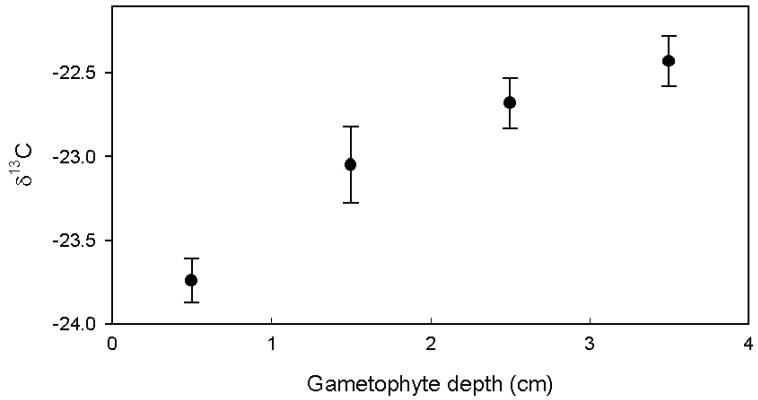


Figure 4: Schematic to show the relative size and densities of gametophyte shoots in desiccated turf of *Grimmia antarctici, Bryum pseudotriquetrum* and *Ceratodon purpureus* within a turf. Note gametophytes have been scaled by 66% in order to avoid overlap, real densities are thus higher than shown for all species.

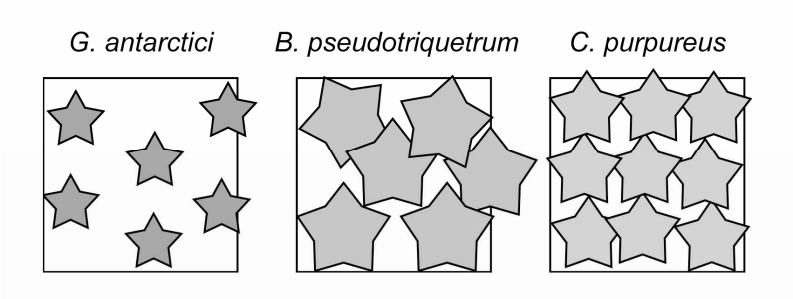
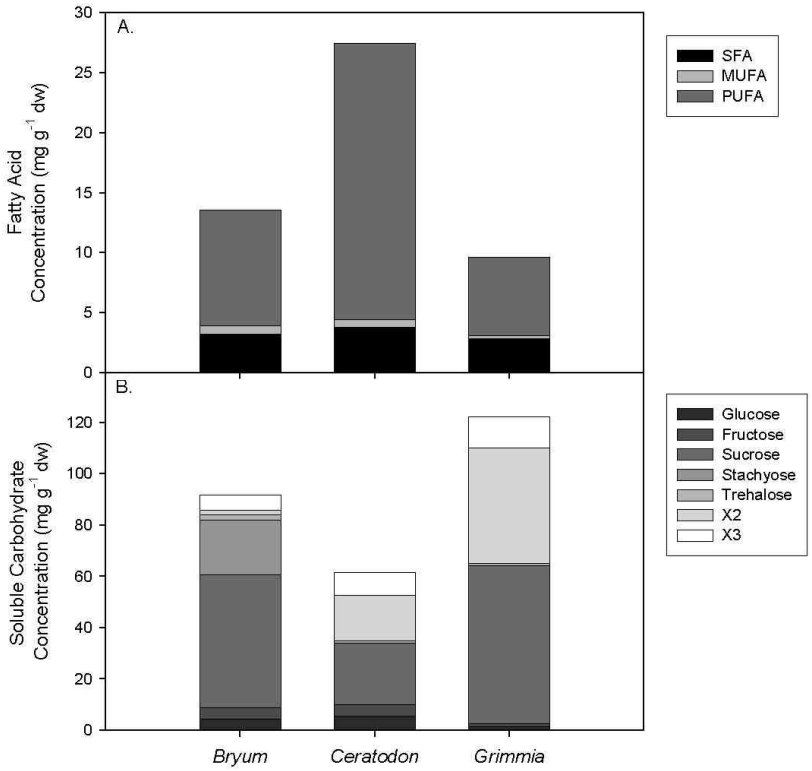


Figure 5: Mean fatty acid and soluble carbohydrate composition (mg g^{-1} dw) for *Bryum pseudotriquetrum*, *Grimmia antarctici* and *Ceratodon purpureus*. Stacked categories (A) indicate proportion of saturated (SFA), monounsaturated (MUFA) and polyunsaturated (PUFA) fatty acids (total concentration SEM is 1.4, 2.9 and 1.2 for the three species respectively, n = 3). Stacked categories (B) indicate proportion of soluble carbohydrates that occur at concentrations > 2 mg g^{-1} dw (total concentration SEM is 2.1, 2.3 and 2.3 for the three species respectively, n = 8).



Tables

Table 1: Summary of 2-way ANOVA results

Results are shown for tolerance of desiccation and submergence variables (critical water content, critical recovery time, submergence ΦPSII) and total soluble carbohydrates, testing for difference between the three species (*B. pseudotriquetrum*, *C. purpureus* and *G. antarctici*), measurement intervals (time) and species * time interactions.

Variable	Transformation	Test	DF (model, error)	F Ratio	Prob > F
Critical Water Content	None	Whole Model	8, 50	9.3006	<0.0001
		Time	2, 50	2.8956	0.0646
		Species	2, 50	18.2650	<0.0001
		Time * Species	4, 50	7.8908	<0.0001
Critical Recovery Time	None	Whole Model	8, 30	31.9097	<0.0001
		Time	2, 30	39.8003	<0.0001
		Species	2, 30	36.9061	<0.0001
		Time * Species	4, 30	28.6313	<0.0001
Submergence ΦPSII	Square	Whole Model	20, 105	27.0044	<0.0001
		Time	6, 105	71.6390	<0.0001
		Species	2, 105	35.7740	<0.0001
		Time * Species	12, 105	3.2256	0.0006
Total soluble carbohydrates	None	Whole Model	8, 57	52.8409	<0.0001
		Time	2, 57	36.3488	<0.0001
		Species	2, 57	147.7512	<0.0001
		Time * Species	4, 57	13.8552	<0.0001

Table 2: Summary of 1-way ANOVA results

Results are shown for shoot width, shoot density, $\delta^{13}C$ (species and long gametophyte) and fatty acids (total, SFA, MUFA, PUFA), testing for difference between the three species (*B. pseudotriquetrum*, *C. purpureus* and *G. antarctici*), and changes in $\delta^{13}C$ along long *G. antarctici* gametophytes. No data transformations were used.

Variable	DF (model, error)	F Ratio	Prob > F
Shoot width	2, 21	20.0948	<0.0001
Shoot density	2, 18	22.0628	<0.0001
$\delta^{13}C$ (species)	2, 21	228.5490	<0.0001
$\delta^{13}C$ (long gametophyte)	3, 16	10.9915	0.0004
Total fatty acids (mg g^{-1} dw)	2, 6	23.0608	0.0015
SFA (mg g^{-1} dw)	2, 6	2.1716	0.1952
MUFA (mg g^{-1} dw)	2, 6	22.3461	0.0017
PUFA (mg g^{-1} dw)	2, 6	20.9052	0.0020

Table 3: Summary of species response to desiccation and submergence

Summary of desiccation and submergence response characteristics for the three study species (*B. pseudotriquetrum*, *C. purpureus* and *G. antarctici*). Quantifying statements indicate the relative response of the species in this study.

Stress Type	Characteristic	*B. pseudotriquetrum*	*C. purpureus*	*G. antarctici*
Desiccation	Avoid	High	High	Low
	Tolerate	Flexible	High	Low
	Survive	Sugars	Lipids	Sugars
	Recover	Fast-Slower	Fast-Slower	Fast
Submergence	Tolerate	High	Low	Highest
	Degree habitat	Low	Low	High

Appendices

Appendix 1: Fatty acid content of the three moss species

Fatty acid composition of *B. pseudotriquetrum*, *C. purpureus* and *G. antarctici* (mg^{-1}g dw). Values in parentheses indicate sem, n = 3. Lipids are grouped into three types: Saturated Fatty Acids (SFAs), Monounsaturated Fatty Acids (MUFAs) and Polyunsaturated Fatty Acids (PUFAs). Only fatty acids detected in concentrations of >0.1 mg^{-1}g dw are listed. Additional fatty acids detected in concentrations of <0.1 only include: 12:0, 14:0, 14:1, 15:0, 15:1, 16:1, 17:1, 20:0, 22:0, 22:1, 24:0, 24:1

Fatty acid			*B. pseudotriquetrum*	*C. purpureus*	*G. antarctici*
SFA	10:0	Capric	1.85 (0.58)	2.04 (0.13)	2.01 (0.07)
	16:0	Palmitic[b]	1.12 (0.11)	1.51 (0.16)	0.67 (0.05)
	18:0	Stearic[b]	0.08 (0.01)	0.12 (0.01)	0.08 (0.01)
MUFA	18:1	Oleic[b]	0.41 (0.03)	0.42 (0.04)	0.12 (0.03)
	18:1	Elaidic (trans)	0.12 (0.01)	0.13 (0.01)	0.05 (0.01)
PUFA	18:2	Linoleic[b]	4.71 (0.98)	7.4 (0.83)	1.33 (0.08)
	18:3 n-6	γ-Linolenic	0.22 (0.07)	0.28 (0.03)	0.03 (0.00)
	18:3	Linoleic	3.37 (0.66)	7.09 (0.72)	4.02 (0.74)
	20:2	Dienoic	0.05 (0.01)	0.37 (0.05)	0.04 (0.00)
	20:3 n-6	Dihomo-γ-linolenic	0.37 (0.08)	0.13 (0.02)	0.03 (0.01)
	20:4	Arachidonic	0.89 (0.01)	1.17 (0.12)	0.43 (0.04)
	20:3 n-3	Eicosatrienoic	0.00 (0.00)	5.45 (0.63)	0.12 (0.02)
	20:5	Eicosapentaenoic	0.04 (0.00)	0.98 (0.10)	0.61 (0.14)
	22:5	Docosapentaenoic	0.00 (0.00)	0.23 (0.01)	0.00 (0.00)

[b]common principal constituents of membrane fatty acids others mainly storage

Appendix 2: Soluble carbohydrate content of the three moss species

Soluble carbohydrates detected in *B. pseudotriquetrum*, *C. purpureus* and *G. antarctici* samples collected over the 1999/2000 summer season (Early; Dec, Mid; Jan and Late; Feb). Data represent mean±se (n = 6-8). Carbohydrates detected at levels <2 mg g^{-1} dw (glycerol, mannitol and *myo*-Inositol) are not shown.

Soluble carbohydrate (mg g^{-1} dw)	Experiment	*B. pseudotriquetrum*		*C. purpureus*		*G. antarctici*	
		Mean	Se	mean	Se	Mean	se
Sucrose	Early	143.72	14.49	29.69	2.94	115.80	10.14
	Mid	44.07	5.86	22.51	2.96	91.22	9.69
	Late	51.66	2.31	23.86	1.41	61.48	1.13
Fructose	Early	8.44	0.70	5.27	0.95	9.10	0.57
	Mid	7.84	0.90	2.49	0.26	5.80	0.40
	Late	4.47	0.79	4.45	0.51	1.30	0.02
Glucose	Early	7.75	0.78	5.59	1.10	8.64	1.12
	Mid	5.11	0.77	3.11	0.26	6.68	0.66
	Late	4.51	1.00	5.75	0.59	1.41	0.03
Stachyose	Early	35.92	3.37	n.d.	n.d.	n.d.	n.d.
	Mid	27.26	3.85	n.d.	n.d.	n.d.	n.d.
	Late	21.31	0.64	n.d.	n.d.	n.d.	n.d.
Trehalose	Early	2.20	1.14	1.21	0.35	0.01	0.00
	Mid	2.73	0.40	2.37	0.21	1.40	0.27
	Late	2.13	0.15	0.79	0.14	1.02	0.10
X2	Early	5.78	0.47	13.08	1.22	42.72	3.91
	Mid	2.33	0.18	18.52	2.05	69.97	8.21
	Late	1.91	0.11	17.80	1.44	44.85	1.45
X3	Early	35.96	2.95	10.51	0.54	23.44	2.53
	Mid	6.25	0.88	8.84	0.97	21.06	2.27
	Late	5.91	0.43	9.01	0.42	12.24	0.18
X4	Early	31.69	3.26	n.d.	n.d.	n.d.	n.d.
	Mid	40.19	5.48	n.d.	n.d.	n.d.	n.d.
	Late	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Total	Early	273.32	26.54	66.48	4.45	200.84	17.68
	Mid	137.09	16.78	58.16	6.33	196.91	20.90
	Late	93.62	2.10	63.10	2.28	123.82	2.34

n.d. indicates not detected