Climate change manipulations show Antarctic flora is more strongly affected by elevated nutrients than water

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**Abstract**
Climate change is expected to affect the high latitudes first and most severely, rendering Antarctica one of the most significant baseline environments for the study of global climate change. The indirect effects of climate warming, including changes to the availability of key environmental resources, such as water and nutrients, are likely to have a greater impact upon continental Antarctic terrestrial ecosystems than the effects of fluctuations in temperature alone. To investigate the likely impacts of a wetter climate on Antarctic terrestrial communities a multi-season, manipulative field experiment was conducted in the floristically important Windmill Islands region of East Antarctica. Four cryptogamic communities (pure bryophyte, moribund bryophyte, crustose and fructicose lichen-dominated) received increased water and/or nutrient additions over two consecutive summer seasons. The increased water approximated an 18% increase in snow melt days (0.2 °C increase in temperature), whilst the nutrient addition of 3.5 g N m⁻² yr⁻¹ was within the range of soil N in the vicinity. A range of physiological and biochemical measurements were conducted in order to quantify the community response. Whilst an overall increase in productivity in response to water and nutrient additions was observed, productivity appeared to respond more strongly to nutrient additions than to water additions. Pure bryophyte communities, and lichen communities dominated by the genus Usnea, showed stronger positive responses to nutrient additions, identifying some communities that may be better able to adapt and prosper under the ameliorating conditions associated with a warmer, wetter future climate. Under such a climate, productivity is overall likely to increase but some cryptogamic communities are likely to thrive more than others. Regeneration of moribund bryophytes appears likely only if a future moisture regime creates consistently moist conditions.

**Keywords**
nitrogen, phosphorous, chlorophyll, stable isotopes, ?13C, chlorophyll fluorescence, bryophytes, lichens, ecophysiology, community ecology

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Climate change manipulations show Antarctic flora is more strongly affected by elevated nutrients than water.

CLIMATE CHANGE AND ANTARCTIC FLORA

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Abbreviations: Abundance of $^{13}$C stable isotope relative to $^{12}$C ($\delta^{13}$C), Abundance of $^{15}$N stable isotope relative to $^{14}$N ($\delta^{15}$N), Antarctic Specially Protected Area (ASPA), Dry weight (dw), Electron Transport Rate (ETR), Nitrogen (N), Phosphorous (P), Turf Water Content (TWC).
Abstract

Climate change is expected to affect the high latitudes first and most severely, rendering Antarctica one of the most significant baseline environments for the study of global climate change. The indirect effects of climate warming, including changes to the availability of key environmental resources, such as water and nutrients, are likely to have a greater impact upon continental Antarctic terrestrial ecosystems than the effects of fluctuations in temperature alone. To investigate the likely impacts of a wetter climate on Antarctic terrestrial communities a multi-season, manipulative field experiment was conducted in the floristically important Windmill Islands region of East Antarctica. Four cryptogamic communities (pure bryophyte, moribund bryophyte, crustose and fructicose lichen-dominated) received increased water and/or nutrient additions over two consecutive summer seasons. The increased water approximated an 18% increase in snow melt days (0.2 °C increase in temperature), whilst the nutrient addition of 3.5 g N m$^{-2}$ yr$^{-1}$ was within the range of soil N in the vicinity. A range of physiological and biochemical measurements were conducted in order to quantify the community response. Whilst an overall increase in productivity in response to water and nutrient additions was observed, productivity appeared to respond more strongly to nutrient additions than to water additions. Pure bryophyte communities, and lichen communities dominated by the genus *Usnea*, showed stronger positive responses to nutrient additions, identifying some communities that may be better able to adapt and prosper under the ameliorating conditions associated with a warmer, wetter future climate. Under such a climate, productivity is overall likely to increase but some cryptogamic communities are likely to thrive more than others. Regeneration of moribund bryophytes appears likely only if a future moisture regime creates consistently moist conditions.
Introduction

High latitudes are predicted to be the first environments to be affected by the onset of climate change (IPCC 2001). Climate change in these environments is expected to result not only in elevated surface temperatures, but also increased precipitation (IPCC 2001; Ye & Mather 1997). Rising temperatures are likely, at least in the short term, to cause additional, indirect increases in moisture availability in areas receiving meltwater from permeant snow and ice reserves (Robinson et al. 2003). As temperature and moisture are key regulators of soil microbial activity (Swift et al. 1979) the projected increases for these variables are likely to produce elevated nutrient availability via increased rates of decomposition and nutrient mineralisation. These indirect effects of climatic change are suggested to be of greater influence to plant productivity than that of temperature alone (Chapin 1983; Hobbie & Chapin 1998; Delucia et al. 2003).

The impact of these climate change effects on continental Antarctic terrestrial communities is not well understood. However, we know from Arctic studies that manipulation of environmental variables associated with climate change, such as water and/or nutrient availability, cause significant impacts on communities (Chapin et al. 1995; Molau & Alatalo 1998; Press et al. 1998a, 1998b; Sandvik & Heegaard 2003). Antarctic terrestrial communities currently survive some of the most extreme growth conditions on Earth and are likely to be sensitive to shifts in these conditions.

Water and nutrient availability are known to play an important role in the present composition of Antarctic terrestrial communities, rendering alterations to their availability highly likely to cause community-level impacts. The low Antarctic temperatures cause most water to be biologically unavailable, in the forms of snow and ice, creating desert conditions. Water availability, is therefore thought to be the primary limiting factor for life on this frozen continent (Kennedy 1993), and has been shown to be the primary determinant of vegetation
patterns at both broad- (Broady 1989; Melick et al. 1994) and fine-scales (Ryan & Watkins 1989; Selkirk & Seppelt 1987), with bryophytes generally showing greater sensitivity to water availability than lichens (Leishman & Wild 2001). Patterns of nutrient availability also play a role in determining vegetation patterns in continental Antarctica (Hovenden & Seppelt 1995; Ryan & Watkins 1989), particularly with respect to lichens and microalgae (Davey & Rothery 1992; Hovenden & Seppelt 1995; Leishman & Wild 2001).

Evidence of past environmental shifts can be observed in the current vegetation structure of the Windmill Islands region of East Antarctica which is dominated by moribund, lichen-encrusted bryophytes that are thought to be indicative of a wetter past (Melick & Seppelt 1997). Long-term climatic drying of this region has promoted lichen expansion and the contraction of bryophytes to only the wettest, low-lying habitats with reliable moisture supply. Melick & Seppelt (1997) demonstrated, via a growth trial, that this shift in vegetation structure might be reversible under conditions of increased water availability. In the present paper, we describe a manipulative field experiment designed to test how increased water and nutrient availability affects terrestrial communities in continental Antarctica. Four community types, which occur along an environmental gradient, from bryophyte-dominated habitat at the edge of a meltlake, to lichen-dominated habitat on the side of a ridge, were investigated.

The four experimental communities received water and/or nutrient additions over two consecutive summer seasons. Our main objectives were to determine if: (1) elevated water and/or nutrient availability will affect primary production, (2) water additions will have a greater impact than nutrient additions, (3) the current ambient availability of water and nutrients differs between community types and (4) some communities respond more strongly than others to water and/or nutrient additions.

As accurate predictions for future water and nutrients are not available for this location our water and nutrient treatments were chosen to fit within the range of those currently found in
the region. Water and nutrient availability to plant communities is currently very patchy (Beyer & Bölter 1998; Wasley 2004). In this study the water treatment was equivalent to an increase in snow melt days from 39-46 days (18%) over the summer season. The nutrient treatment was within the range for current soil nutrients in the vicinity (Beyer & Bölter 1998). The overall aim of the study was to provide an insight into how Antarctic terrestrial communities are likely to respond to climate change, under a warmer, wetter climate scenario. We predict that water and nutrient additions will increase plant primary productivity in these communities. Based on the overall emphasis in the literature, we further predict that plants will respond more strongly to water-additions than nutrient-additions. The experimentally manipulated communities are likely to respond differently to water and nutrients and show variation in the strength and nature of their response to these additions. This experiment is one of the only multi-season manipulative field experiments that have been conducted in continental Antarctica (Robinson et al. 2003). It therefore provides a particularly important contribution to our understanding of how plants in this environment are likely to respond to climate change.

Materials and Methods

Study Area

The Windmill Islands region in East Antarctica is a 40 by 15 km area, consisting of a series of ice-free islands and peninsulas, topographically characterised by low rounded hills of altitudes generally less than 100 m. The climate is dry, with the rainfall equivalent of mean annual snowfall less than 200 mm yr\(^{-1}\). Temperatures are cold, with approximate mean monthly temperatures ranging from 0 °C in the warmest summer months to –15 °C in the winter. Extremes of temperature range from approximately – 40 to +10 °C. Gale force winds are common, annually averaging one day in four (Melick & Seppelt 1994).
In the past, the region has been completely ice-covered (Hollin & Cameron 1961) and ice sheet thickness during the Pleistocene is estimated to have been 200 m (Goodwin 1993). The region became deglaciated during the Holocene, with the south exposed first, 8000 years ago, and the north a few thousand years later, by 5500 years ago (Goodwin 1993). Since deglaciation, the land has isostatically uplifted 53 m (Goodwin 1993). As a result of this uplift, penguin rookeries, which were once close to the sea, have been abandoned as newly exposed sites closer to the water are occupied. Abandoned penguin colonies are now common around the 30 m contour, and are frequently colonised by lichens.

The Windmill Islands is a botanically important region, supporting some of the most extensive and best-developed vegetation on continental Antarctica. The entirely cryptogamic vegetation was first described by Smith (1988), who determined that the region supports at least 27 species of macrolichens and four bryophytes. Three bipolar lichens dominate the vegetation *Usnea sphacelata* (Hoffm.) Th. Fr., *Umbilicaria decussata* (Vill.) Zahlbr. and *Pseudephebe minuscula* (Nyl. Ex Arnold) Brodo & Hawksw., which is typical for continental Antarctica (Smith 1988).

The bryoflora is restricted to low-lying, moist sites, and is composed of three moss species (*Grimmia antarctici* Card. ≈ *Schistidium antarctici* Card., *Bryum pseudotriquetrum* (Hedw.) Gaertn., Meyer & Scherb. and *Ceratodon purpureus* (Hedw.) Brid.) and one liverwort (*Cephaloziella exiliflora* (Tayl.) Steph.). Of these species, the Antarctic endemic *G. antarctici* is most abundant, while the other two moss species are of widespread cosmopolitan distributions but locally less abundant. The distribution of these species is thought to be determined by water availability, as *G. antarctici* is restricted to the wettest sites, *C. purpureus* occupies the driest of sites and *B. pseudotriquetrum* co-occurs with both *G. antarctici* and *C. purpureus* (Selkirk & Seppelt 1987; Wasley 2004). The liverwort, *C.
*exiliflora* has a southern Australasian and scattered Antarctic distribution (Seppelt 1983) and locally is most commonly found co-occurring with *C. purpureus*.

The experimental site was located in the Antarctic Specially Protected Area (ASPA) 135 on Bailey Peninsula, approximately 1 km east of Casey Station. Within ASPA 135, the site was situated on the western edge of a meltlake, close to the ASPA 135 northern boundary, at the top of a snow slope above Thala valley (66° 16.03’ S, 110° 32.53’ E). Site aspect was northeasterly and the site was in the vicinity of an abandoned penguin rookery (Fig. 1). The site spanned a bryophyte community gradient running up the ridge away from the meltlake; with pure bryophyte stands at the lake edge, through a moribund bryophyte zone to the point at which the bryophyte community is predominantly moribund and dominated by crustose lichen species. This community gradient is thought to occur along a moisture gradient, and is indicative of a drying trend, along which live bryophyte communities have retreated to low-lying wet habitats with reliable moisture supply (Melick & Seppelt 1997). Water supply for these communities is mainly from melting of overlying snow, which transiently saturates the plants. However, the moss community on the lake edge remains waterlogged for most of the growing season.

**Experimental design**

Within the experimental site, four community types were identified based on the percentage cover of four key community components: healthy bryophytes, moribund bryophytes, crustose lichens and macrolichens of the genus *Usnea* (details of community composition are given in Fig. 1). The Bryophyte and Crustose communities correspond to Group 1 and 2.3, respectively, in Melick & Seppelt (1997). The Moribund community is an intermediate community. The *Usnea* community corresponds with Group 2.5 (Transitional, Foliose lichen-dominated, *Usnea*; Melick and Seppelt 1997). This latter community is considered to be the
earliest indicator of the presence of extant bryophyte communities, which thrived when conditions were moister.

The four communities occurred along a gentle slope, with an easterly aspect, between the meltlake edge and the side of a small ridge. The Bryophyte community occurred closest to the meltlake, and the other three communities were positioned with increasing distance from the meltlake edge, in the order of: Moribund community, Crustose community and, furthest from the meltlake, the *Usnea* community, which was closest to the ridge (Fig. 1).

Within each community, 32 equivalent quadrats (25 x 25 cm) were randomly assigned one of the following four treatments: (1) no-treatment (NT-), (2) water only (W-), (3) nutrient only (N-), or (4) water & nutrient (WN-). The two water addition treatments (W- and WN-) had 500 ml of meltlake water applied approximately every two days across the 1998/99 and 1999/00 summer seasons (December - February). To avoid beading of the applied water off the vegetation surfaces, and therefore maximise the effectiveness of the water additions, each quadrat was first pre-soaked using a fine, firm, even spray of water from a pressurised spray bottle. The remaining treatment water was then slowly and evenly poured over the area of the quadrat. The water treatment was intended to alleviate water limitation by saturating the turf at each application. The 500 mL additions were excess to that absorbed and simulated an increase in snow melt days from 39 to 46, equivalent to a 0.2 °C increase in temperature over the summer months (Dunn & Robinson *pers. comm.*). Since all three moss study species grow in areas which become periodically saturated this was a realistic treatment assuming either increased snowfall or increased melt. The water was taken from the meltlake adjacent to the site; as this is a natural water source of the bryophyte communities at the height of the summer melt.

Quadrats receiving nutrient additions (N- and WN-) had 10 g of slow release fertiliser beads (Osmocote, Scotts Australia Pty. Ltd., Castle Hill, NSW, Australia) applied at the start of the
treatment period (15/12/98). The Osmocote was composed of 18% nitrogen (7.5% as nitrate, 
10.5% as ammonium), 4.8% phosphorous (4.3% water soluble, 0.5% citrate soluble), 9.1% 
potassium (as potassium sulphate), 4.0% sulphur (as sulphate) and 1.0% calcium phosphate 
and calcium sulphate, with a 9.1% organic resin coating. These additions constituted 
approximately 3.5 g N m\(^{-2}\) yr\(^{-1}\) and 1 g P m\(^{-2}\) yr\(^{-1}\), which is comparable to additions used in 
Arctic climate change simulation studies (Chapin et al. 1995; Molau & Alatalo 1998), and 
low relative to the N storage of Windmill Islands’ soils (21-736g N m\(^{-2}\); Beyer & Bölter 1998). This type of fertiliser treatment was chosen to achieve a slow release of nutrients over 
the duration of the study period, avoiding both nutrient “spikes” and the need to reapply 
nutrients with multiple applications. Beads were inserted into the bryophyte turf and/or lichen 
crust with tweezers to ensure nutrient additions remained within relevant treatment quadrats. 
Empty beads were recovered at the end of the study period, indicating that the fertilizer 
treatments had remained in place.

**Primary productivity surrogates**

In other high-latitude ecosystems, ecological measures of community change (such as, 
biomass and species cover) have been successfully used to monitor response to environmental 
manipulations (Press et al. 1998b; Robinson et al. 1998). The characteristically slow growth 
rates that occur in continental Antarctica (Green 1985), however, limit studies of this nature to 
utilising physiological methodologies. Instead of using direct measures of growth, such as 
biomass, a variety of physiological measurements were used as surrogates for primary 
productivity and the growth environment.

Chlorophyll fluorescence, chlorophyll and plant nutrient content and stable isotope ratios 
were measured for representative vegetation within each community type. Samples from the 
Bryophyte, Moribund and Crustose communities consisted of well-developed bryophyte turf, 
increasingly moribund and encrusted with crustose lichens in the Crustose community.
Samples from the *Usnea* community consisted of only a thin bryophyte crust and were dominated by lichens, predominantly from the genus *Usnea*.

**Field measurement of chlorophyll fluorescence**

Chlorophyll \(a\) fluorescence is particularly useful for measuring photosynthesis in Antarctic cryptogams, since their small size makes gas exchange measurements difficult (Green *et al.* 1998; Lovelock *et al.* 1995a, 1995b) and such non-destructive methods are ideal where plant conservation is a major concern. Although electron transport rate (ETR) is not a direct measure of photosynthetic gas exchange, simultaneous fluorescence and gas exchange measurements have shown that fluorescence ETR measurements correlate directly with CO\(_2\) gas exchange in the moss species *Bryum argenteum* (Schroeter *et al.* 1995) and *Ceratodon purpureus* (J. Wasley, unpublished data).

Electron transport rates were measured using chlorophyll fluorescence during the main growing season (14-15/2/00). Replicate measurements were taken under consistent light conditions, between 2 & 4 pm, within randomly selected quadrats (n=6) from each community and treatment. Measurements were performed using a MINI-PAM equipped with a micro leaf clip (Walz, Effeltrich, Germany). ETR was calculated using the equation: ETR = \(\phi_{\text{PSII}} \times \text{PAR} \times 0.5 \times 0.84\), as described by Maxwell & Johnson (2000). ETR was used in preference to measures such as the ratio of variable to maximal fluorescence (Fv/Fm), which requires a dark adaptation period. Dark adaptation of plant material was found to be unsuitable in this environment, since plant tissue temperature declined significantly as soon as solar radiation was interrupted.

**Laboratory analysis of chlorophyll, nutrients and stable isotopes**

At the end of the treatment period (3–8/3/00), representative community samples were collected for pigment and nutrient analyses. Seven replicate quadrats were sampled over a 5-day period from each of the sixteen treatment and community groups. Samples of
approximately 4 cm² were collected from the centre of each quadrat, in subsets of 16, each subset including one replicate from each community and treatment type.

Each sample was divided into three; approximately 1 cm² area comprised the chlorophyll pigment and turf water content (TWC) samples for which the surface portion (top 2-3 mm) was reserved for chlorophyll pigment analyses and the base was used to estimate TWC. Of the remaining larger portion (approximately 3 cm²), the top 1 cm was used to determine plant nutrient contents (N and P). For each sample, total chlorophyll, TWC, nutrient (N and P) and stable isotope (δ¹⁵N and δ¹³C) signatures were determined, as described below.

Samples for chlorophyll determinations were frozen in liquid nitrogen, transported to Australia and stored at -80 °C until analysis. Pigments were extracted into 80% acetone from approx 0.02-0.20 g of plant samples and chlorophyll analysed using the method of Porra et al. (1989).

The availability of water in the growth environment was determined for samples of bryophyte turf that were collected from the field in airtight vials. Using an analytical balance, fresh weight was determined immediately upon return to the laboratory. Dry weights were determined after oven drying at approximately 70 °C, till constant weight. The turf water content (TWC) of each moss sample at each time period was determined as described in Robinson et al. (2000, 2005).

Prior to determination of nitrogen, phosphorus, δ¹⁵N and δ¹³C, plant material was oven dried, as described for TWC and ground to a fine powder (0.2 mm particles) in a ball mill (Retsch MM2, Vienna, Austria). Aliquots of 1 to 2 mg of each sample were analysed by continuous-flow gas isotope ratio mass spectrometry after Hietz et al. (1999). The elemental analyzer (EA 1110, CE Instruments, Milan, Italy) was interfaced via a ConFlo II device (Finnigan MAT, Bremen, Germany) to the gas isotope ratio mass spectrometer (DeltaPLUS, Finnigan MAT).
Total phosphorous was determined in duplicate for each sample by HNO₃-H₂SO₄ digestion, followed by stannous chloride colorimetry determined at 690 nm (Clesceri et al. 1998; Spectronic 21D, Spectronic Instruments, Leeds, UK).

In the Usnea community, it was difficult to collect pure vegetation samples that were free of soil contamination, as bryophytes occurred in this community only as a thin moribund crust, rather than a well-developed turf. To calculate nutrient contents on a dry weight basis, ash weights of samples were determined and weight % N and P corrected as described in Wasley (2004).

**Statistical Analysis**

For each of the biological parameters measured, a two-way ANOVA was conducted, testing means for community differences, treatment effects and treatment x community interactions. Transformations of the data were required in some cases to ensure an acceptable level of normality and homoscedacity (see Table 1). Where the treatment x community interaction was significant, post hoc pairwise (Student’s t) tests were conducted for all ecologically relevant pairs of means (i.e. within both treatment and community groups) in conjunction with the Bonferroni procedure (Rice 1989). As the Bonferroni procedure reduces the alpha level, and in turn the chance of identifying significant pairs, it is argued that the Bonferroni procedure can sometimes cause biologically important difference to be overlooked (Moran 2003). For this reason, any additional pairs of means that are significant at $\alpha = 0.05$ are reported. The results of these pairs must be interpreted with caution, but are worth noting, as they are potentially biologically significant. Statistical tests were conducted using JMP software (SAS Inc., Cary, NC, USA).
Results

Photosynthetic efficiency

Photosynthetic ETR was similar in the control group (NT-) across the communities, and all communities showed a positive response to the water and/or nutrient additions (Fig. 2A). Several notable community differences were found, as the strength and nature of the treatment response differed across communities (treatment x community interaction: $P = 0.0003$; Table 1). The treatment response was weak (not significant) in the Moribund and Usnea communities, and strongest in the Crustose and Bryophyte communities. The strongest response to treatments was found in the Crustose community, where ETRs in all treatment groups were greater than 4-fold higher than the untreated control ($P<0.002$). This community responded more strongly to the addition of water than nutrients, as the two water-treatments (W- and WN-) had significantly higher ETRs than the nutrient-only treatment ($P<0.002$ and 0.05, respectively). Conversely, the Bryophyte community, which also showed a significant treatment response, responded most strongly to nutrient additions, with ETRs highest in the two nutrient treatments where rates were more than 2-fold higher than the untreated control (N- $P<0.05$, WN- $P<0.002$). In this community the effect of water alone was not significant ($P>0.05$). Although the moribund community response was not significant, all treatments resulted in 2-fold higher ETRs than untreated samples.

Total chlorophyll

Nutrient additions had a positive effect on concentration of total chlorophyll, particularly in the Bryophyte and Crustose communities (treatment effect: $P<0.0001$; Table 1) where total chlorophyll concentrations were 2- to 3-fold greater in the two nutrient-treatment groups (N- and WN-), compared to the corresponding non-nutrient treated groups (NT- and W-; community effect, $P<0.0001$; Table 1; Fig. 2B). The Moribund and Usnea communities showed a relatively weak treatment response. Concentration of total chlorophyll also differed
between the communities, with Crustose having the highest and Moribund the lowest overall concentrations ($P<0.008$). Chlorophyll a/b ratios, which ranged from approximately 1.5 to 3.0, showed significant differences between communities, but there was no effect of treatment. Ratios were highest in the lichen-dominated Crustose and Usnea communities ($P<0.008$).

**Water, nutrient and stable isotope composition**

**Turf water content**

Turf water content (TWC; g $\text{H}_2\text{O}$ g$^{-1}$ dry wt.) ranged from just less than 1 to slightly over 2 g $\text{H}_2\text{O}$ g$^{-1}$ dry wt. (Fig. 3A). Water and/or nutrient additions had no significant effect on TWC, but there was significant variation between the communities (Table 1), with the Crustose and Bryophyte communities having higher TWCs than the relatively dry Moribund and Usnea communities ($P<0.008$ and 0.05, respectively).

**Nutrients**

Nutrient additions (N-) enhanced weight %N in all communities except the Moribund community (overall model: $P < 0.0001$; treatment x community interaction: $P = 0.0340$; Table 1; Fig. 3B). In all three communities showing this response, the nutrient-only additions (N-) produced %N levels that were significantly higher than both the non-nutrient treated groups (NT- and W-, $P<0.002$ and 0.05, respectively). In the Crustose community, nutrient only additions also elevated %N significantly more than the combined water and nutrient additions ($P<0.002$). The Usnea community was the only community in which the combined water and nutrient treatment (WN-) enhanced %N significantly over levels in untreated control samples (NT-, $P<0.05$).

Elevated water and/or nutrient availability did not significantly alter %P, but significant community differences for this variable were identified (Table 1). Percent P showed a strong community gradient (community effect: $P<0.0001$; Table 1), with a greater than 20-fold
increase from the Bryophyte to the *Usnea* community ($P<0.008$; Fig 3C). Percent N was also lowest in the Bryophyte community ($P<0.002$), and generally highest in the Crustose community, where control levels (NT-) were significantly higher than all communities except Moribund ($P<0.002$).

**Stable Isotopes**

Water and/or nutrient additions did not significantly alter stable isotopic signatures for $^{15}$N or $^{13}$C (Table 1), indicating the fertilizer did not significantly affect $\delta^{15}$N, nor did the watering regime significantly alter $\delta^{13}$C (Fig. 3D). There was, however, significant community variation in stable isotopic signatures (community effect: $P = <0.0001$; Table 1). Variation between communities was primarily due to distinctions in $\delta^{13}$C, rather than $\delta^{15}$N (data not shown). The most notable community distinction found for $\delta^{15}$N were the Crustose community values, which were significantly more positive (13.6±1.9‰, $n=6$ to 17.4±1.9‰, $n=5$) than the other three communities (6.2±0.9‰ to 11.7±2.4‰, $n=7$; $P<0.008$). Much stronger community differences occurred in $\delta^{13}$C signatures, for which each community was significantly different to the others ($P<0.008$), with values highest (least negative) in the Bryophyte community and lowest (most negative) in the *Usnea* community (Fig. 3D).

**Discussion**

On this desert continent, water is considered to be the most limiting environmental parameter to plant life (Robinson *et al.* 2003, Wasley *et al.* 2006), more so even than the direct limitations associated with the low temperatures that dominate this ecosystem. It was therefore expected, that increased water availability, would be most influential and that the addition of nutrients, which are generally not considered limiting (Robinson *et al.* 2003), would have a secondary, but possibly synergistic, effect. This study shows a positive response to increased water availability, but surprisingly, a strong positive response to nutrient additions was also found. Background availability of the two manipulated environmental
variables varied across the site, producing differences in availability of these resources between the communities. Thus natural variation in resource availability, likely influenced the differences in treatment-response that were found across the communities.

The water and nutrient treatments used in this study were designed to alleviate water and/or nutrient limitations, with quantities applied within the range currently found in the region. The rationale for this approach is two-fold, firstly to investigate biological response under non-limiting conditions and secondly due to the difficulties associated with predicting future water and nutrient availability and patchiness. In the Antarctic environment, precipitation levels are difficult to measure due to interference with blowing snow and ice across the landscape. This renders estimates of present water availability to plant communities difficult and makes modelling of future availability unreliable for application to this type of study. Similarly, the increase in future nutrient availability will remain difficult to quantify until more certainty is developed regarding future temperature conditions and water availability.

However, global warming is likely to increase thawing depth, releasing N and P from the permafrost (Beyer & Bölter 1998). Nutrient inputs from bird sources are important to present vegetation patterns (isotopic data presented here, Leishman & Wild 2001; Ryan & Watkins 1989) and changes to this nutrient source will also impact on future nutrient availability.

This discussion focuses on three of the main findings of this study. Firstly, we discuss gradients in water and nutrient availability across the site and their relationship to plant community types. Secondly, community differences in treatment-response are discussed, which support the hypothesis that some communities would respond more strongly than others to water and/or nutrient additions. Finally, we discuss how this study offers support for the hypotheses that elevated water and/or nutrient availability affects primary production surrogates, but does not support the hypothesis that water additions will have a greater impact than nutrient additions.
Environmental gradients

The ambient availability of water and nutrients differed between the communities. These differences were largely determined by two opposing environmental gradients across the site, one a gradient in water availability, the other a gradient in nutrient availability (Wasley 2004).

Water availability

As the site was situated on the edge of a meltlake, a moisture gradient occurred across the site, with the Bryophyte community occupying the lowest-lying, wettest habitat and the Usnea community the highest and driest (Fig. 1). Community patterns of $\delta^{13}C$ support the presence of this gradient in water availability. More positive $\delta^{13}C$ values are indicative of less photosynthetic isotopic fractionation, resulting from diffusional limitations reducing the concentration of CO$_2$ reaching the site of RUBISCO. In higher plants this diffusional fractionation relates to stomatal opening. However in cryptogams it likely reflects the degree to which plants are submerged in water and the consequent diffusional limitations (Bottger et al. 1993; Proctor et al. 1992; Rice & Giles 1996). Cryptogams subject to submergence are expected to show elevated (less negative) $\delta^{13}C$ signatures, as has been shown in Sphagnum trinitense (Proctor et al. 1992). The relatively high $\delta^{13}C$ found for the Bryophyte community in this study (control: -22.7±0.1‰, n=6) represents a cumulative signature, reflecting a tendency for periodic submergence during the summer melt. The $\delta^{13}C$ community patterns therefore likely reflect a submergence gradient, and field observations strongly support the occurrence of periodic submergence in the Bryophyte community during the growing season.

Values for $\delta^{13}C$ provided stronger support for the presence of a moisture gradient occurring across the site than did turf water contents. It is likely, however, that for a single measurement interval $\delta^{13}C$ is a better integrator of plant moisture availability over the season, as it provides cumulative evidence of submergence. Turf water content is an instantaneous measurement and does not necessarily reflect water availability for the majority of the growth season. We
suggest that the anomaly between TWC and $\delta^{13}$C is not due to the lack of change in water availability over the site, but instead reflects the timing of sample collection and site topography. Samples were collected late in the season, by which time the level of water in the meltlake had dropped and the water had frozen; leaving the Bryophyte community relatively dry and at a seasonally low level. Moreover, sample collection occurred after snowfall, and snow accumulation across the site therefore resulted in elevated moisture in the other communities at that time. These factors, combined, explain the lack of evidence for a moisture gradient presented in the TWC data.

Site topography is likely to have contributed toward the relatively high moisture contents found in the Crustose community and $\delta^{13}$C data suggest that this represents the second most submerged community (Fig. 3D). This community was situated on a relatively flat, terraced section in the middle of the site (Fig. 1), causing the snowfall to accumulate on this community. We therefore suggest, that the turf water content data was collected at a time when the Bryophyte community was drier than usual and the Crustose community wetter than usual, and is not necessarily representative of the water contents of these communities for the majority of the growing season. Field observations, $\delta^{13}$C values and data from adjacent sites supporting comparable communities provide strong evidence that a submergence gradient is present across this site for the majority of the growth season (Wasley et al. 2006).

**Nutrient availability**

Fractionation of nitrogen isotopes provides information about plant nitrogen sources (BassiriRad et al. 2003; Bragazza et al. 2005). Antarctic plants commonly have high $\delta^{15}$N, due to utilisation of nutrients derived from marine animal sources such as penguins (Cocks et al. 1998a, 1998b; Robinson 2001). Although there are few present day inputs at the study site, an abandoned penguin rookery was located at the top of the low rocky ridge above the site. Such abandoned rookeries are known to provide a significant nutrient source to plants in
Antarctic terrestrial communities (Hovenden & Seppelt 1995; Tatur & Myrcha 1989). Nutrients deposited when these rookeries were occupied, approximately 5000 years ago (Goodwin 1993), undergo further $^{15}N$ fractionation as the guano passes through additional tropic levels associated with decomposition. Plants utilising this ancient nitrogen source are expected to have exceptionally high $\delta^{15}N$ values higher than +14.6‰, the value for fresh penguin guano (Robinson 2001). Lower $\delta^{15}N$ values indicate plants are receiving a higher proportion of volatilised wind-born NH$_3$ from present-day rookeries (Robinson 2001) and/or cyanobacterial N fixation. The high $\delta^{15}N$ values found in plant material in this study indicate that the nutrient source for this community is likely to be the nearby abandoned penguin rookery. Soil in the vicinity of the site also had high $\delta^{15}N$ (23.84±1.18‰, n=6; Wasley 2004). We suggest this rookery provides a significant nutrient-source for the site and underlies the gradient in nutrient availability observed across the site.

Plant nutrient content was highest in communities closest to the ridge and declined with distance from the rookery, confirming the rookery as the major nutrient-source for the site (Fig. 3B&C). This pattern was particularly strong for phosphorous. Substratum nutrient concentrations in the Hovenden & Seppelt (1995) abandoned rookery site match the patterns found in the present study; phosphorous declined 15-fold within 25 m from the rookery, while nitrogen showed a less sharp decline, of less than 2-fold, over the same distance.

Site hydrology is also likely to interact with plant nutrient contents. The nearby lake drains into a stream that flows out of the site. Snow melt moving down the slope, from the abandoned rookery on the ridge, to the lake is likely to be a source of nutrients, but the wetter community at the edge of the lake (i.e. Bryophyte community) is likely to be heavily leached as nutrients flow out of the catchment. The terrace upon which the Crustose community is positioned might act to sequester nutrients for a longer period than on the steeper slopes and/or the periodically submerged lakeside positions.
Community response to water and nutrient additions

Differences in treatment-response were found for the four experimentally manipulated communities, supporting the hypothesis that some communities would respond more strongly than others to water and/or nutrient additions. Overall, the Bryophyte and Crustose communities showed a strong treatment-response, while the Moribund and Usnea communities responded weakly.

The Bryophyte community responded most strongly to nutrient additions in both primary productivity surrogates. The Crustose community similarly showed a strong increase in total chlorophyll in response to nutrient additions but in contrast, ETR responded more strongly to water additions. Some of these community differences in treatment-response were likely to be related to the resource availability gradients that were observed across the site. The Bryophyte community, for example, in which water contents are usually highest, responded most strongly to nutrients. While the Crustose community, in which water availability is estimated to be relatively low for much of the growing season, showed a response to both treatments.

Although not significant, there are indications that the treatments increased productivity in the Usnea and Moribund communities. Such a limited response was particularly unexpected for the Moribund community. This community is thought to have formed in response to a drying trend, and it was predicted that lichen encrusted moribund moss, which characterises this community, may have shown regeneration potential in response to water additions, as this has been shown to occur under controlled growth conditions (Melick & Seppelt 1997). It is possible that the water treatment was not sufficient for moss regeneration, but it may also be that the encrusting lichen species were responsible for this lack of recovery through allelopathy. Species of the genus Buellia are commonly found encrusting moss in this study area and it has been suggested that this species exhibits allelopathic characteristics (Melick & Seppelt 1997). It is therefore possible, that the allelopathic effects of lichens or the action of
antagonistic fungi (Melick & Seppelt 1994) were responsible for the limited response of this community. This finding is particularly important since it suggests that changes to water availability alone may not be sufficient to reverse the trend of drying climate producing moribund moss.

Live bryophyte material is present in the Crustose and Moribund communities (Wasley 2004), it is likely, however, that only conditions of consistent soaking would allow them to regenerate. If this is the case, regeneration will only be likely under a future climate that produces increased frequency and intensity of water availability. Otherwise, it is likely that crustose lichens will continue to expand in distribution, encrusting bryophyte turfs.

**Impacts on primary productivity surrogates**

Although the hypothesis that elevated water and/or nutrient availability will affect primary productivity surrogates is supported, the hypothesis that water additions will have a greater impact than nutrient additions is not. The impacts of water and/or nutrient additions are summarised below.

**Response to water additions**

The strongest response to water additions was found in the Crustose community, where photosynthetic rates increased more than 7-fold in response to the watering treatment (Fig. 2A). Turf water contents suggest that, at the time of sample collection, water availability was relatively high in this community (Fig. 3A). Field observations and δ¹³C values suggest, however, that this community was drier than the Bryophyte community, for the majority of the growth season, explaining why the response to water-treatment was less pronounced in the Bryophyte community. Although the Crustose community showed a nutrient response (see below) the water response was stronger. Weaker than expected responses to water additions have also been observed in temperate grassland bryophyte communities (Bates et al. 2005).
Response to nutrient additions

Despite the general suggestion that nutrients are non-limiting to Antarctic plant physiological processes (Robinson et al. 2003), this study found strong responses to nutrient additions, supported by a 3-fold increase in total chlorophyll concentration and 4-fold increase in ETR in the Bryophyte and Crustose communities, respectively.

Plant nitrogen increased significantly in response to the nutrient-only additions in all but the Moribund community, with the most striking increases occurring in the Usnea community where %N almost doubled (Fig. 3B). These nutrient increases indicate sufficient natural moisture availability within most communities for nutrient uptake, and that the nutrient treatments were effective. With increased replication it seems likely that the nutrient treatments would deliver significantly higher productivity parameters in all communities, including Usnea and Moribund.

Increasing ETR, in response to the water and nutrient additions, suggests that the plants are limited by these environmental factors and have the ability to respond by increasing photosynthesis. If increases in the concentration of N or chlorophyll had been the only response this might have indicated the plants simply absorb the nutrients and sequester them, but are in fact limited by other factors in terms of photosynthetic rate. These results support increased photosynthetic productivity as a result of nutrient additions. Further long-term studies would be required to show if growth was positively affected by nutrients.

It has previously been suggested that sufficient nitrogen is available in precipitation (Greenfield 1992) but the nutrient response observed in this study suggests these communities may be experiencing nutrient-limitations. Although soil nutrients are quite high in this region (Beyer & Bölter 1998), they may not be readily available to cryptogams which lack roots. Other examples of nutrient-limitation in Antarctic terrestrial communities have been reported, including nutrient-limited growth of lichens situated away from bird colonies (Ryan &
Watkins 1989), nitrate limitation of microalgae in fellfield soils in the maritime Antarctic (Davey & Rothery 1992; Arnold et al. 2003) and nutrient-limited bryophyte carbon assimilation in the sub-Antarctic (Smith 1993). In milder and subarctic sites, climate warming is predicted to reduce bryophyte and lichen cover as a result of poor nutrient competiveness against vascular plants (Cornelissen et al. 2001; Epstein et al. 2004; Wahren et al 2005) whereas in the high arctic bryophytes also respond positively to nutrient additions (Robinson et al. 1998; van Wijk et al. 2004). This study concurs with these latter studies suggesting that nutrient-limitations may be widespread in both Antarctic and high Arctic systems (Jonasson 1992; Robinson et al. 2004; van Wijk et al. 2004). This study does not allow us to determine which nutrients are limiting, and it is possible that nitrogen, phosphorus, or a combination of nutrients, are responsible for these strong nutrient treatment responses.

**Synergistic effects**

Some evidence that combined water and nutrient additions had a synergistic effect on plant community response was found in this study. The strongest examples of synergism were observed in the rates of photosynthetic electron transport in the Bryophyte and Usnea communities, where although some increases were observed in response to water and nutrient additions when applied singularly, the highest rates occurred when both water and nutrients were added (Fig. 2A). Also, a trend toward synergism was observed for total chlorophyll in the Crustose and Usnea communities, where total chlorophyll concentrations were highest in the combined water and nutrient treatments (Fig. 2B). This result was surprising since it shows that sufficient water is available for most communities - except perhaps the driest, Usnea - to take up additional nutrients when they are available, rather than water plus nutrients being required to elicit this response.
Conclusions

This study represents one of the only manipulative field experiments to be conducted in continental Antarctica, and illustrates both that such studies are possible and can provide valuable information regarding the way in which Antarctic terrestrial communities are likely to respond to climate change. Primary productivity surrogates showed an overall increase in response to water and nutrient additions, but appeared to respond more strongly to nutrients than to water. Certain communities are likely to thrive under a wetter future, provided nutrient availability increases with increasing water availability. Increased productivity was most pronounced in the Crustose and Bryophyte communities, suggesting these community types may be most able to adapt and prosper under the ameliorating conditions associated with a warmer, wetter future climate. However, if nutrient availability does not increase along with wetter conditions, bryophyte communities may be nutrient-limited and fail to thrive.

Acknowledgements

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References


### Table 1: Summary of 2-way ANOVA results for total chlorophyll concentration (Total Chl), electron transport rate (ETR), turf water content (TWC), % Nitrogen (%N), % Phosphorous (%P) and nitrogen and carbon stable isotopic signatures ($\delta^{15}$N and $\delta^{13}$C).

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<th>Variable</th>
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<th>Test</th>
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Figure Legends

Figure 1: Position within the site and distinguishing characteristics of the four communities types used in the field water and nutrient enrichment experiment. The meltlake is to the left and the abandoned rookery ridge to the right of the main photo. Percent cover was estimated using a 25x25 cm quadrat. Grimmia antarctici is the dominant bryophyte species across site.

Figure 2: Influence of the four water and nutrient treatments on (A) photosynthetic electron transport rate (ETR; μmol m⁻² s⁻¹) and (B) total chlorophyll concentration (nmol g⁻¹ dw) for plant material from the four experimentally manipulated communities (Bryophyte, Moribund, Crustose and Usnea) after two seasons treatment with water, nutrients, water plus nutrients or no treatment. Bars represent mean ± sem, n = 6 (A) n = 7 (B).

Figure 3: Influence of the four water and nutrient treatments on (A) turf water content (TWC; g H₂O g⁻¹ dw), (B) percent N (C) percent P and (D) δ¹³C (‰) signature, for plant material from the four experimentally manipulated communities (Bryophyte, Moribund, Crustose and Usnea). Samples were collected after 15 months treatment with water, nutrients, water plus nutrients or no treatment. Bars represent mean ± sem, n = 5-7. Note y-axes scales are different.
Figures

Figure 1:
Figure 2:
Figure 3:

(A) TWIC (g 11.0 g dW)

(B) % N

(C) % P

(D) δ¹³C (%o)

Communities:
- Bryophyte
- Mcribium
- Crustose
- Usnea

Legends:
- Nc+ Treatment
- Water
- Nutrient
- Water & Nutrient