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Surface reflectance properties of Antarctic moss and their relationship to plant species, pigment composition and photosynthetic function

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Keywords

Bryum pseudotriquetrum, Ceratodon purpureus, chlorophyll, chlorophyll fluorescence, Grimmia antarctici, Photochemical Reflectance Index, xanthophyll cycle pigments

Disciplines

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Surface reflectance properties of Antarctic moss and their relationship to plant species, pigment composition and photosynthetic function

RUNNING TITLE: Surface reflectance of moss

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Abstract

We investigated how surface reflectance properties and pigment concentrations of Antarctic moss varied over species, sites, microtopography, and with water content. We found that species had significantly different surface reflectance properties, particularly in the region of the red edge (approximately 700 nm), but this did not correlate strongly with pigment concentrations. Surface reflectance of moss also varied in the visible region and in the characteristics of the red edge over different sites. Reflectance parameters, such as the Photochemical Reflectance Index (PRI) and Cold Hard Band (CBH) were useful discriminators of site, microtopographic position and water content. The PRI was correlated both with the concentrations of active xanthophyll-cycle pigments and the photosynthetic light use efficiency, F_v/F_m, measured using chlorophyll fluorescence. Water content of moss strongly influenced the amplitude and position of the red-edge as well as the PRI, and may be responsible for observed differences in reflectance properties for different species and sites. All moss showed sustained high levels of photoprotective xanthophyll pigments, especially at exposed sites, indicating moss is experiencing continual high levels of photochemical stress.

Keywords: *Bryum pseudotriquetrum*, *Ceratodon purpureus*, chlorophyll, chlorophyll fluorescence, *Grimmia antarctici*, Photochemical Reflectance Index**,** xanthophyll cycle pigments

Introduction

Solar radiation is essential for photosynthesis and growth of plants. The surface reflectance characteristics of leaves is key in determining both the quantity and quality of solar radiation that is incident on leaf internal organs (Vogelmann 1993). Although solar radiation in the visible region drives photosynthesis, absorptance of high levels of visible radiation and radiation at other wavelengths can be damaging. At shorter wavelengths, absorbed UV-B (280-320 nm) radiation can cause lesions to nucleic acid and proteins. Excess levels of visible radiation (400-750 nm) can cause photoinhibition of photosynthesis (Cornic, Woo & Osmond 1982). High absorptance at longer wavelengths (>750 nm) leads to increases in temperature that can be detrimental in arid environments where it is important for plants to conserve water by limiting transpiration and to maintain leaf temperatures close to that of air temperatures (Gates *et al.* 1965; Ehleringer 1981). Variation in absorptance of leaves has been observed (Ehleringer & Björkman 1978; Vogelmann 1993) and specialized adaptation of surface reflectance properties in plants is known to facilitate photosynthetic carbon gain. For example, in desert ecosystems pubescent or waxy leaf surfaces that increase reflectance are common (Billings & Morris 1951, Ehleringer 1981) and have been demonstrated to be important in protection from photoinhibition of photosynthesis (e.g. Robinson, Lovelock & Osmond 1993). Moreover, epidermal tissues often accumulate compounds that are effective screens against UV-B radiation, thereby decreasing their reflectance in the UV region (e.g. Tevini 1993).

In this study we surveyed the natural variability of surface reflectance in moss in continental Antarctica. At our study site in Wilkes Land, eastern Antarctic, conditions are cold and usually very arid, resulting in lichens dominating the rocky, ice-free areas. The moss

community is well developed for the region, but it is restricted to melt lakes, melt streams and other locations where free water is available for at least some of the summer (November-March). In winter the moss is covered with snow and ice and is subject to disturbance from cryo-perturbation, which causes highly complex microrelief of ridges and valleys within the moss turves. Compared to lower latitudes, levels of UV radiation are low in Antarctica. But, Antarctica is experiencing large increases in incident UV-B radiation due to reductions in concentrations of stratospheric ozone (Frederick & Snell 1988). Increases in UV radiation are having large effects on the primary production of the oceans (Neale, Davis & Cullen 1998), and could also be affecting terrestrial vegetation. The heat balance of moss, photosynthetic processes and the impacts of enhanced UV radiation are likely to be partially dependent on moss surface reflectance characteristics and pigment composition. Thus, the first aim of this study was to test the hypothesis that moss species and moss inhabiting different environments have different surface reflectance characteristics. We measured surface reflectance at approximately one nm intervals from 200-900 nm of three dominant moss species over three sites with differing water availabilities, and over differences in microtopography.

Surface reflectance properties of vegetation are often used as indicators of photosynthetic function in remote sensing of vegetation (reviewed in Field, Gamon & Penuelas 1994). Variation in reflectance has been observed among plant taxa (e.g. Gates *et al.* 1965; Penuelas *et al.* 1993; Gamon *et al.* 1995), due to differences in water and nutrient availability (e.g. Penuelas *et al.* 1994; Gamon, Serrano & Surfus 1997), environmental stress (e.g. Rock, Hoshizaki & Millar 1988), and both seasonally (Running & Nemani 1988; Gamon *et al.* 1995) and diurnally (Gamon *et al.* 1990; Penuelas *et al.* 1994; Gamon *et al* 1997). Changes in surface reflectance of intact leaves have been directly correlated with leaf chemical composition (Jacquemoud *et al.*

1996) and changes in photosynthetic processes in some higher plant taxa (Gamon *et al.* 1990; Gamon *et al.* 1997, Gilmore & Ball 2000). But it is often difficult to reconcile physiological measurements, which are often made at the scale of individual leaves and remotely sensed measurements, which are often made at the scale of whole canopies (Williams 1991; Field *et al*. 1994). Some of the difficulty arises because canopies are complex. Canopies are composed of layers of leaves of different species that can have a range of properties. Leaves within canopies can have varying distributions, orientations, morphologies, internal structures, pigment compositions, and epidermal characteristics, all properties that could influence the surface reflectance of the canopy (Rock *et al.* 1988; Field *et al.* 1994;Vose *et al*. 1995). At the leaf level, mosses are very simple consisting of a few cell layers. But at a higher level of organization, moss turves, although small in comparison to canopies of trees, are relatively complex, composed of many leafy gametophytes packed together to form what can be thought of as a 'micro-canopy'. In mosses, measurement of photosynthetic processes and surface reflectance can be made at the same scale. Thus, the second aim of our study was to test the hypothesis that spectral properties of moss are directly related to photosynthetic processes and pigment composition. We wished to understand whether non-destructive hyperspectral measurements could potentially be used to map moss species distributions and physiological activity both spatially and temporally. Here we describe tests of correlations among surface reflectance properties and commonly used reflectance indices and pigment concentrations, water contents and photosynthetic function of Antarctic moss.

Materials and methods

Moss was obtained from three sites around the Australian base Casey, Wilkes Land, continental Antarctica (66º17' S, 110º32' E). Two sites were directly adjacent to the base (Red Shed and Science), while the other site was 20 km west at Robinson Ridge. Water availability varies across the sites. The Red Shed site is directly adjacent to a large summer melt lake, the Robinson Ridge site is adjacent to a melt stream that flows intermittently over the summer months, while at the Science site water is only available when overlying snow melts early in the summer. Temperature also varies over the sites. Robinson Ridge is cooler, and more exposed, than the Red Shed and Science sites (Melick, Hovendon & Seppelt 1997). In the early 1990s the Science site was exposed to contamination due to deposition of cement dust during construction (Adamson, Adamson & Seppelt 1994).

At each site representative samples, approximately 2 cm^2 were cut from the turves over 3 summer seasons (October to February) from 1996 to 1999. The three most common species, *Grimmia antarctici* Card., *Ceratodon purpureus* (Hedw.) Brid., and *Bryum pseudotriquetrum* (Hedw.) Gaertn., Meyer and Scherb. were sampled. The necessity of minimizing impacts of the study, the extent of the moss beds (smaller at Science) and the natural distribution of the three moss species resulted in different representations of each species at each site (Table 1). We tested for differences between moss species and the influence of site on pigment and surface reflectance parameters using samples from all sites. We used a subset of the Robinson Ridge and Red Shed *Grimmia antarctici* samples to test for effects of microtopography.

Additionally we tested the influence of moss water content on surface reflectance. We used 4 samples of *G. antarctici* from microtopographic ridges at Robinson Ridge. We measured

the surface reflectance of the samples when they were dry, and after they had been rehydrated with a fine mist of water for 5 minutes. Excess water was blotted from the moss before measurements. Moss re-hydrate rapidly, regaining full physiological activity in one to two minutes (Robinson et al. 2000).

Measuring surface reflectance

We used an integrating sphere fitted to a scanning spectrophotometer (GBC UV-Vis 918, GBC Australia) to measure spectral reflectance of moss between 200 – 900 nm at approximately 1 nm intervals on 2 cm^2 areas of moss canopy. The moss turf samples were large enough to completely fill the sample aperture of the integrating sphere. To maintain moss canopy architecture (gametophyte arrangement) the samples were held in place by within a 1cm deep highly reflective sample holder. For some samples this required shaving tissue from the basal portion of the sample using a razor blade until the turf was 1 cm thick. The depth and packing of the samples resulted in transmittance of through the sample being negligible. Reflectance measured therefore represents the moss canopy with green photosynthetic tissue of between 2-5 mm depth and 8-5 mm of non-photosynthetic tissue below. From the entire reflectance spectra, using subsets of the total 87 spectra (Table 1), we present mean reflectance at key wavelengths and also calculate reported indices used in remote sensing vegetation (Table 2). The Cold Hard Band (CHB, Gilmore & Ball 2000) has been found to correlate with the formation of a chlorophyll-protein complex in leaves that protects against freezing damage. The Photosynthetic Reflectance Index (PRI, Gamon *et al.* 1990, 1997) was developed to reflect changes in concentrations of the xanthophyll cycle pigments that are formed when plants are stressed. The amplitude of the reflectance change at the red-edge ($_{RE}$) and the position of the red-edge (λ_{RE})

were calculated from the first derivative of the spectra (Horler, Dockray & Barber 1983). Other reflectance indices used by a range of researchers were also calculated (e.g. Vogelmann, Rock & Moss 1993; Gitelson & Merzylak 1997; Lichtenthaler, Gitelson & Lang 1996; Carter 1991, 1993).

Pigment, water and photosynthetic characteristics

Prior to measurement of the reflectance spectra, a subset of the *G. antarctici* samples were dark adapted for 20 minutes after which the chlorophyll fluorescence parameter F_v/F_m was measured using a PAM 2000 (H. Walz, Effeltrech, Germany). After the reflectance spectra were measured, the photosynthetically active apices of the moss were removed using a razor blade and the tissue was frozen in liquid nitrogen. Samples were returned to Australia in liquid nitrogen and then stored at –80 ˚C in a freezer prior to pigment analysis. For chlorophyll and carotenoid determination, samples (50-100 mg fresh weight) were ground with liquid nitrogen and sand in a mortar and pestle and then extracted in (1.5 ml) 100% acetone. The samples were transferred to a micro-centrifuge tube containing 1 mg sodium bicarbonate and kept on ice in the dark for 20 minutes. After centrifugation (13,000 rpm for 5 minutes) the pellet was re-extracted in 0.5 ml 80% acetone using a polypropylene tissue grinder (Crown Scientific, Australia). After a further 20 minutes on ice, the sample was centrifuged as above and the supernatants from each extraction combined and made up to 3 ml with 100% acetone. Chlorophylls and carotenoids were then quantified by high-pressure liquid chromatography (HPLC) using a method adapted from Gilmore & Yamamoto (1991). The samples (30-70 µl) were injected into a Shimadzu HPLC system (Shimadzu, Australia) at a flow rate of 2 ml min^{-1}. Solvent A

(acetonitrile:methanol:Tris HCL buffer 0.1 M, pH 8.0; 79:8:3) ran isocratically from 0 to 4 min followed by a 2.5 min linear gradient to 100% Solvent B (methanol:hexane; 4:1) which then ran isocratically from 6.5 -15 min. Flow rate was decreased from 2 to 1.5 ml min^{-1} between 6-12 min and then ran at 1.5 ml min^{-1} until 15 min, to maintain stable pressure. The column was re-equilibrated with solvent A between samples. Pigments were separated on an Spherisorb ODS1 column (Alltech, Australia) and quantified by integration of peak areas, detected at 440 nm using a photodiode array detector (Model SPD-M10AVP, Shimadzu, Australia), relative to pure chlorophyll (Sigma, Australia) and carotenoid (Extrasynthase, France and VKI, Denmark) standards.

Anthocyanin concentrations were determined using the differential pH method of Fuleki & Francis (1968) and Francis (1982). Moss tissue (0.1-0.2 g fresh weight) was ground with liquid nitrogen and sand in a mortar and pestle and then extracted in (1.5 ml) of 1% HCl in methanol. Samples were centrifuged at 13,000 rpm for 6 min and absorbance of the supernatant measured at 526nm. 100 µl of sodium acetate buffer (pH 5.0) was then added to 1 ml of the supernatant and after 3 min this sample was centrifuged for 10 min at 13,000 rpm before a second measurement of absorbance at 526 nm. Anthocyanin concentration was calculated from the difference in these two absorbance measures.

Water content of samples was measured after oven drying at 80 ˚C to stable weight and is expressed as gH_20 $gdryweight^{-1}$ as described in Robinson *et al.* (2000). Concentration of UV-B absorbing pigments was determined from 15 to 60 mg of dry moss tips. Dry tissue was weighed, ground in liquid nitrogen with acid-washed sand and then extracted in 1.5 ml of acidified methanol (methanol-H_2O-HCl; 79:20:1). The sample was transferred to a microfuge tube and allowed to stand on ice in the dark, with vortexing every 20 min. After 1 hour the tube was

centrifuged (13,000 rpm, 4 min) and the supernatant removed. The final volume of supernatant was made up to 1.5 ml with acidified methanol. Absorbance across the UV-B region (280-320nm) was scanned spectrophotometrically. Values are expressed as mean absorbance for this wavelength range.

Data analysis

Correlations among surface reflectance indices, pigments, chlorophyll fluorescence parameter F_v/F_m and water content were tested using the non-parametric Spearman Rank Correlation (*Rho*) using the statistical computing package Data Desk 6.1 (Data Descriptions 6.1, Ithaca NY). *Rho* detects consistently increasing or decreasing trends but does not assume variables are linearly related. Linear regression was also used to test for significant relationships between moss chlorophyll concentrations and reflectance indices, and among other variables. Tests of the influence of site, species and microtopography on pigments and reflectance parameters were performed using analysis of variance (ANOVA). For tests of sites and species ANOVAs were unbalanced (unequal sample sizes), therefore post-hoc tests of differences between individual means were adjusted for the unequal sample sizes using the Bonferroni test. The adequacy of the ANOVA models was assessed by inspecting residual plots. Paired t-tests were used to assess the influence of hydration on reflectance parameters.

Results

Differences between species in reflectance and pigments

Reflectance spectra of mosses were similar in shape to those observed for intact leaves of angiosperms with the major features of the spectra those indicative of chlorophyll (Fig 1). Examination of the 1st derivative of the spectra (Fig 1B) showed typical peaks at the green (approximately 525 nm) and red edge (approximately 700 nm). Moss species did not significantly differ in their UV reflectance (R320), but they differed significantly at 526, 550 and 850 nm, in both $_{RE}$ and $_{RE}$, and tended to have different CHB (Table 3). In all cases, *B. pseudotriquetrum* had greater reflectance values.

Pigment concentrations also varied among species (Table 3). *Bryum pseudotriquetrum* had higher levels of UV-absorbing pigments but lower carotenoid levels (total carotenoid and xanthophyll concentrations) compared to *C. purpureus* and *G. antarctici*. *Ceratodon purpureus* had lower total chlorophyll concentrations than *G. antarctici* or *B. pseudotriquetrum* but higher levels of anthocyanins.

Many of the moss pigments concentrations tended to co-vary with each other. For example, total chlorophyll concentration was highly correlated with the total carotenoid content (*Rho* = 0.91). Additionally, concentrations of all the carotenoids tended to be highly correlated (e.g. correlation among lutein and the xanthophyll cycle pigments *Rho* = 0.726). Anthocyanins, measured as absorptance of extracts at 526 nm and expressed on a fresh wt basis, were also correlated with total chlorophyll (*Rho* = 0.625). The proportion of xanthophyll-cycle pigments in

the de-epoxidated photoprotective forms, zeaxanthin and antheraxanthin, was negatively correlated with total chlorophyll (*Rho* = -0.482) and positively correlated with the total pool size of the xanthophyll-cycle pigments (*Rho* = 0.588).

Microtopography

The PRI was strongly influenced by whether moss was growing on a ridge or valley, but no other reflectance parameter was significantly affected by microtopographic position (Table 4). Samples from ridges had lower values of PRI (more negative) than those in the valleys. Total chlorophyll concentrations were approximately half on the ridges compared to the valleys, while the concentration of xanthophyll-cycle pigments per chlorophyll was 50% higher. There was also a significantly higher proportion of the xanthophyll-cycle present in the photoprotective forms on ridges.

Sites

Over the three sites we observed significant difference in many of the reflectance parameters, but there were no significant effects of site on R320, PRI, NDVI or NPCI (Table 5). The Science site had higher reflectance at 526 and 550 nm, but lower reflectance at 850 nm compared to moss growing at the Red Shed and Robinson Ridge sites. Moss at the Red Shed site exhibited greater $_{RE}$ and $_{RE}$ values than did moss at the Robinson Ridge and Science sites. The CHB was also higher at Robinson Ridge than at the other two sites. Moss at the Red Shed site had lower EGFR and EGFN than moss at Robinson Ridge and Science.

Differences in reflectance indices corresponded to changes in pigment contents over the sites. Total chlorophyll and anthocyanin concentrations were not significantly different among sites, although mosses tended to have higher concentrations of these pigments at Robinson Ridge than at Science and Red Shed sites (Table 5). Xanthophyll pigment concentrations, total carotenoids and concentrations of UV absorbing pigments were all greater at Robinson Ridge than at the other two sites.

Relationships among reflectance, plant pigments, water content and photosynthesis

Overall, correlation between surface reflectance indices and plant pigment contents was low. Concentration of total chlorophyll was correlated significantly, but not strongly with many of the key reflectance wavelengths and indices (Table 6). The strongest linear regression was between total chlorophyll and reflectance indices calculated with reflectance measured at 850 and 680 nm (either R850, R850-680, and NDVI) and with $_{RE}$. There was no significant relationship between total chlorophyll and EGFR, EGFN or NPCI.

The surface reflectance characteristic of moss that correlated most strongly with the concentration of extracted anthocyanin pigments measured at 526 was $_{RE}$ (*Rho* = 0.426). The PRI, a tool developed to remotely assess plant stress (Gamon *et al.* 1997) was negatively correlated with both the total pool size of the xanthophyll-cycle pigments (*Rho* = -0.402) and also the proportion as zeaxanthin and antheraxanthin (Fig 3A, *Rho* = –0.535). The PRI was positively correlated with the chlorophyll fluorescence parameter, F_v/F_m (Fig 3B, *Rho* = 0.626). Declines in F_v/F_m indicate declines in the efficiency of photosystem II.

To assess the influence of water content on reflectance parameters we rehydrated dry samples of *G. antarctici* in the laboratory. Re-hydration resulted in a significant increase in $_{RE}$ accompanied by a shift in $_{RE}$ to shorter wavelengths (Table 7). Re-hydration also resulted in a significant increase in the PRI and a decrease in the CHB (Table 7).

Discussion

Species

We found that moss species, moss at different sites, and moss from different microtopographic positions varied significantly in their surface reflectance properties, but different reflectance parameters were sensitive to different ecological or physiological factors (Table 8). The parameters most sensitive to species and site factors were reflectance in the visible region (R526 and R550), and the features of the red-edge ($_{RE}$ and $_{RE}$). Among moss species, *B. pseudotriquetrum*, had higher values of reflectance indices than other species (Fig. 1, Table 3). This was not simply associated with differences in chlorophyll concentrations, which were higher than those of *C. purpureus* but similar to that measured in *G. antarctici*. *Bryum pseudotriquetrum* is morphologically distinct from the other two species, having much larger gametophytes with larger leaves that are densely packed into the turf (S.A. Robinson, unpublished data). Gametophyte size is similar in the other two species, but gametophytes of *C. purpureus* are also densely packed, whereas those of *G. antarctici* plants are more loosely arranged within the turves. Reflectance parameters are likely to be influenced by gametophyte morphology and packing independently of chlorophyll concentrations. Reflectance from

canopies is sensitive to the leaf area index (LAI; Booches *et al.* 1990; Penuelas *et al.* 1994; Serrano, Gamon & Penuelas 2000) and to other features of canopy geometry e.g. leaf thickness, surface quality and orientation (Rock *et al.* 1988). Thus, differences observed in reflectance characteristics among moss species could be more strongly influenced by differences in plant morphology (e.g. gametophyte morphology, density and thickness of photosynthetic tissue) than by physiological factors. It should be noted that *B. pseudotriquetrum* is the species that shows the most 'angiosperm leaf like' reflectance. Unfortunately, since moss surface reflectance has not been well studied we are unable to say if *B. pseudotriquetrum* or the other species are most typical of moss plants.

In previous work we have found that these three moss species desiccate at different rates and can maintain photosynthetic function at different levels of desiccation (Robinson *et al.* 2000). Given the sensitivity of the red-edge features to water content (Table 7), differences among reflectance of moss species and sites could also be due to variation in water contents.

Pigment composition also varied between species with *B. pseudotriquetrum,* having higher levels of specific UV –screening pigments whilst *C. purpureus* and *G. antarctici* showed higher levels of pigments generally associated with photoprotection from excess visible light. In the latter species the ratio of xanthophyll cycle pigments/chlorophyll was high and characteristic of plants exposed to much higher levels of solar radiation. Given that samples for pigment analysis were not frozen until several hours after collection, the sustained de-epoxidation status of the xanthophyll cycle pool is also indicative of relatively high photochemical stress in these plants. Differences in relative pigment concentrations between the three species also suggests that they may be differentially vulnerable to increasing UV-B exposure as a result of ozone depletion.

Microtopography

Over different microtopographic positions *G. antarctici* samples on ridges had lower PRI than samples from valleys, but no differences were observed in any other reflectance parameters, despite large differences in chlorophyll concentrations between moss on ridges and valleys. In other studies higher chlorophyll concentrations and proportion of green tissue are associated with shifts in $_{RE}$ to longer wavelengths (Curran *et al.* 1992; Vogelmann *et al.* 1993; Serrano *et al.* 2000). The lack of difference in the features of the red-edge, or other reflectance indices despite large differences in chlorophyll concentrations over ridges and valleys suggest that $_{RE}$ and $_{RE}$ are largely independent of chlorophyll concentrations over the range found in this study. In both ridges and valleys moss turves form closed micro-canopies that likely have high 'leaf' area indices (LAI). In higher plants, canopies with high LAI are often not distinguishable using reflectance parameters (Booches *et al.* 1990; Penuelas *et al.* 1994; Serrano, Gamon & Penuelas 2000). Thus, the dense packing of gametophytes in moss may contribute to the insensitivity of reflectance parameters to differences in microtopography.

Lower PRI on ridges than valleys was associated with higher concentrations of xanthophyll-cycle pigments and greater de-epoxidation of xanthophyll cycle pigments; characteristics of plants experiencing conditions that limit photosynthetic carbon gain and result in photoinhibition (Osmond 1994; Björkmann & Demmig-Adams 1995; Lovelock, Osmond & Seppelt 1995a; Lovelock *et al.* 1995b). Moss on ridges is exposed to much higher levels of solar radiation than moss shaded in microtopographic valleys. Moss on ridges also experience lower and more variable water availability. Higher light levels in combination with lower water

contents are likely to lead to photoinhibition, de-epoxidation of xanthophyll cycle pigments and reduced PRI.

Sites

Those reflectance indices sensitive to site but not species were EGFN, EGFR and CHB. In our assessment of reflectance parameters over sites we expected that sites with lower water availability (Robinson Ridge and Science) would have differences in reflectance parameters and pigment concentrations that were indicative of greater exposure to environmental stress. Our prediction was largely supported with EGFN and EGFR being higher at the drier Science and Robinson Ridge sites, than at the Red Shed site (Table 6). Differences in EGFN and EGFR are products of the differences in the red edge over sites, where the amplitude of the red edge is steeper and at longer wavelengths at the drier sites. The CHB was greater at the cooler western Robinson Ridge site than at Science and Red Shed, which corresponded to the lower average temperatures recorded at Robinson Ridge (Melick *et al.* 1997).

Total chlorophyll concentrations were greater at Robinson Ridge than at the other sites. Because we did not observe differences in $_{RE}$ and $_{RE}$ due to the microtopographic position of moss, where chlorophyll concentrations were vastly different, we conclude that changes in $_{RE}$ and $_{RE}$ over sites is due to some other factors than differences in chlorophyll concentrations. Other important factors could be differences in moss species composition over the sites, differences in water content, and/or gametophyte morphology. It is also possible that the higher reflectance at the Science site is also due to the increased presence of fine dust particles on the moss surface as a result of local human activity.

Relationships among reflectance, plant pigments, water content and photosynthesis

Despite sensitivity of surface reflectance characteristics to species, site and microtopography, surface reflectance indices were not strongly correlated with pigment concentrations, with the exception of the correlation between PRI and xanthophyll-cycle pigments. The PRI also correlated with the chlorophyll fluorescence parameter F_v/F_m, a measure of PSII function, as has also been shown by Gamon *et al.* (1997) and others (e.g. Méthy 2000). The NDVI, an index commonly used in broad band remote sensing, was shown to be insensitive to the range of factors assessed in this study (Table 8) as has also been found by others (e.g. Buschmann & Nagel 1993).

Water content of moss appears to be a key parameter influencing $_{RE}$ and $_{RE}$ and PRI. The red edge has been shown to shift to longer wavelengths when bean leaves are infiltrated by water (Knipling 1970). More commonly observed is a 'blue shift' in leaves and plant that are subjected to water stress (Horler *et al.* 1983; Booches *et al.* 1990; Carter 1991, 1993; Penuelas & Inoue 1999), although in some cases measurements are complicated by a reduction in chlorophyll in the water stressed tissues (Maracci *et al.* 1991). Desiccation tolerant mosses show much greater variation in water content than angiosperm leaves (i.e. these species tolerate a 12- 28 fold difference in water content in live tissues, Robinson *et al.* 2000). Thus, a shift toward the red as water content increases in moss may reflect processes that are particular to the physiology of desiccation resistant plants. These processes may be more similar to those involved in greening of angiosperm leaves (e.g. Gates *et al.* 1965; Curren *et al.* 1991; Vogelmann *et al.* 1993) than those as a result of acute environmental stress.

The sensitivity of the surface reflectance indices to species and site and some physiological parameters suggests that mapping of moss communities might be possible using hyperspectral sensors. From these results it seems likely that high spectral and spatial resolution sensor such as the Compact Airborne Spectrographic Imager (CASI), programmed to detect narrow wavebands in the visible and NIR, could potentially differentiate between species and detect changes in physiological activity at small spatial scales (1 m^2 or less, depending on the deployment height of the sensor). These methods have already proven useful in the mapping of aquatic and littoral communities, where the use of visible wavebands was essential (Zacharias *et al,* 1992; Bajjouk, Guillaumont & Poplus 1996, Fyfe and Dekker 2001) and in assessment of photosynthetic activity in terrestrial plant communities (e.g. Zarco-Tejada *et al.* 2000). Hyperspectral remote sensing could provide an effective and nondestructive method of mapping the moss communities that occur in remote Antarctic and other high latitude locations, and to monitor impacts of climate change on the spatial extent and composition of these communities that are highly sensitive to climatic trends (Doran et al. 2001).

Conclusions

In Antarctic moss surface reflectance characteristics vary among species and are significantly influenced by the environmental conditions under which plants grow. Differences in reflectance characteristics across sites and species were not clearly linked to changes in pigment concentrations but maybe more strongly linked to differences in water contents and morphology of gametophytes and turves. The PRI was the surface reflectance index most sensitive to variation in pigment compositions and photosynthetic function. Hyperspectral imaging of plant

communities? may be a useful tool in assessing the impacts of global climate change in Antarctica. Moss may also be a useful 'system' to test the applicability of new parameters for remotely sensing the physiological state of vegetation.

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Table 1. Details of species and site for moss samples used in the study.

Species	Number of Samples per Site		
	Robinson Ridge	Red shed	Science
Ceratodon purpureus	4	4	5
Bryum pseudotriquetrum	7	3	2
Grimmia antarctici	24	36	2
TOTAL	35	43	9

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Table 2. Calculation of reflectance indices

Parameter	Index	Calculation
Cold Hard Band[1]	CHB	(R750-R710)/(R750+R710)
Normalized difference between the first derivative in the red and green[2]	EGFN	(1st R530-1st R710)/(1st R530-1st R710)
Ratio of first derivative in the green to first derivative in the red[2]	EGFR	(1st R530/1st R710)
Normalized difference vegetation index[3]	NDVI	(R850-R680)/(R850+R680)
Normalized pigment chlorophyll ratio index[2]	NPCI	(R680-R430)/(R680+R430)
Photosynthetic reflectance index[4]	PRI	(R531-R570)/(R531+R570)

[1](Gilmore and Ball 2000); [2](Penuelas *et al.* 1994); [3](Rouse *et al*. 1973); [4](Gamon *et al.* 1997)

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Table 3. Surface reflectance characteristics and pigment concentrations for three common moss species in Continental Antarctica, N = 11, 12, 63 respectively. NS signifies a non-significant difference at the *P*=0.10 level).

	Bryum pseudotriquetrum	*Ceratodon purpureus*	*Grimmia antarctici*	*F*	*P*
Reflectance					
R320	0.0070 ± 0.0008	0.0071 ± 0.0010	0.0070 ± 0.0003	NS	
R526	0.049 ± 0.005	0.022 ± 0.006	0.023 ± 0.001	28.40	<0.0001
R550	0.055 ± 0.007	0.034 ± 0.008	0.034 ± 0.002	5.84	0.0074
R850	0.388 ± 0.037	0.275 ± 0.020	0.311 ± 0.012	4.29	0.0170
RE	0.0101 ± 0.0001	0.0060 ± 0.0001	0.0068 ± 0.0001	8.74	0.0004
λRE	705.4 ± 1.9	700.6 ± 1.0	700.4 ± 0.5	7.33	0.0012
PRI	-0.160 ± 0.028	-0.160 ± 0.015	-0.172 ± 0.009	NS	
NDVI	0.835 ± 0.047	0.800 ± 0.029	0.868 ± 0.012	NS	
NPCI	0.442 ± 0.057	0.484 ± 0.043	0.398 ± 0.024		
EGFR	6.04 ± 1.58	8.82 ± 2.43	8.03 ± 0.85	NS	
EGFN	0.643 ± 0.042	0.784 ± 0.072	0.701 ± 0.021	NS	
CHB	0.349 ± 0.058	0.229 ± 0.021	0.261 ± 0.015	2.74	0.0708
Pigments					
Anthocyanins $A_{526\ diff} g^{-1}$ fwt	1.2 ± 0.2	1.3 ± 0.2	1.2 ± 0.2	NS	
Anthocyanins/TChl. $A_{526\ diff} mol^{-1}$	2.9 ± 0.3	5.7 ± 1.2	3.0 ± 0.2	7.42	0.0011
UV absorbing pigments Mean $A_{(320\text{-}280nm)}$ g^{-1} dwt	266 ± 42	127 ± 22	110 ± 9	15.34	<0.0001
Total Chlorophyll nmol g^{-1} fwt	475 ± 60	241 ± 40	453 ± 44	2.50	0.0882
Chl a:b ratio	3.2 ± 0.2	3.6 ± 0.3	3.2 ± 0.1	NS	
VAZ/TChl mmol mol^{-1}	64 ± 5	132 ± 36	103 ± 6	3.64	0.0305
%(AZ/VAZ)	18.0 ± 3.9	39.4 ± 4.9	37.4 ± 2.5	6.37	0.0026
Tcar./Tchl. mmol mol^{-1}	443 ± 15	643 ± 96	631 ± 31	2.86	0.0629

Table 4. Surface reflectance properties and pigment concentrations of *Grimmia antarctici* over different microtopographic positions. Moss is from ridges or valleys at Robinson Ridge and Red Shed sites, *N*=8. NS signifies a non-significant difference at the *P*=0.10 level).

	Ridge	Valley	*F*	*P*
Reflectance				
R320	0.0058 ± 0.0003	0.0066 ± 0.0006	NS	
R526	0.027 ± 0.003	0.0022 ± 0.001	NS	
R550	0.035 ± 0.004	0.042 ± 0.003	NS	
R850	0.362 ± 0.016	0.374 ± 0.023	NS	
RE	0.0079 ± 0.0001	0.0084 ± 0.0001	NS	
λRE	700.75 ±1.26	700.25 ± 1.19	NS	
PRI	-0.213 ± 0.009	-0.117 ± 0.011	48.79	0.0001
NDVI	0.849 ± 0.013	0.882 ± 0.031	NS	
NPCI	0.333 ± 0.064	0.425 ± 0.069	NS	
EGFR	8.90 ± 1.77	9.33 ± 2.16	NS	
EGFN	0.761 ± 0.032	0.780 ± 0.043	NS	
CHB	0.295 ± 0.025	0.256 ± 0.036	NS	
Pigments				
Anthocyanins $A_{526\ diff}g^{-1}$ fwt	1.0 ± 0.4	1.9 ± 0.3	NS	
Anthocyanins/TChl. $A_{526\ diff}mol^{-1}$	2.7 ± 0.5	2.6 ± 0.6	NS	
Total Chlorophyll nmol g^{-1} fwt	375 ± 73	791 ± 66	17.88	0.0008
Chl a:b ratio	3.3 ± 0.2	3.1 ± 0.2	NS	
VAZ/TChl mmol mol^{-1}	130 ± 12	81 ± 6	12.87	0.0030
%(AZ/VAZ)	40.9 ± 5.4	27.1 ± 3.3	4.82	0.0454
Tcar./Tchl. mmol mol^{-1}	761 ± 87	465 ± 18	10.98	0.0051

Table 5. Surface reflectance characteristics and pigment concentrations of moss over three sites in Continental Antarctica. N = 34, 43, 9 respectively. NS signifies a non-significant difference at the *P*=0.10 level).

	Robinson Ridge	Red shed	Science	*F*	*P*
Reflectance					
R320	0.0068 ± 0.0004	0.0070 ± 0.0004	0.0086 ± 0.0011	NS	
R526	0.0027 ± 0.0004	0.0023 ± 0.0004	0.0038 ± 0.0011	4.94	0.0094
R550	0.035 ± 0.002	0.034 ± 0.001	0.052 ± 0.004	3.43	0.0371
R850	0.351 ± 0.017	0.289 ± 0.015	0.331 ± 0.024	4.28	0.0170
$_{RE}$	0.0081 ± 0.0001	0.0063 ± 0.0001	0.0070 ± 0.0001	3.93	0.0235
$_{\lambda RE}$	703.8 ± 1.1	699.3 ± 0.2	700.1 ± 0.4	11.91	<0.0001
PRI	-0.165 ± 0.014	-0.177 ± 0.010	-0.146 ± 0.011	NS	
NDVI	0.857 ± 0.019	0.864 ± 0.015	0.808 ± 0.042	NS	
NPCI	0.454 ± 0.035	0.391 ± 0.027	0.370 ± 0.051	NS	
EGFR	10.19 ± 1.43	5.86 ± 0.58	9.30 ± 2.99	4.62	0.0127
EGFN	0.764 ± 0.032	0.651 ± 0.023	0.742 ± 0.062	4.36	0.0159
CHB	0.324 ± 0.029	0.226 ± 0.008	0.256 ± 0.026	4.52	0.0137
Pigments					
Anthocyanins $A_{526\ diff}g^{-1}$ fwt	1.5 ± 0.2	1.1 ± 0.2	1.3 ± 0.1	NS	
Anthocyanins/TChl. $A_{526\ diff}mol^{-1}$	3.5 ± 0.5	3.3 ± 0.4	3.3 ± 0.3	NS	
UV absorbing pigments Mean $A_{(320\text{-}280nm)}$ g^{-1} dwt	213 ± 37	132 ± 20	104 ± 17	4.29	0.0224
Total Chlorophyll nmol g^{-1} fwt	500 ± 58	374 ± 49	393 ± 39	NS	
Chl a:b ratio	3.2 ± 0.2	3.3 ± 0. 1	3.4 ± 0.2	NS	
VAZ/TChl mmol mol^{-1}	124 ± 14	93 ± 6	61 ± 7	4.72	0.0114
%(AZ/VAZ)	29.7 ± 3.0	40.4 ± 3.0	28.5 ± 6.3	3.68	0.0292
Tcar./Tchl. mmol mol^{-1}	677 ± 55	586 ± 29	458 ± 16	3.19	0.0462

Table 6. Reflectance signals of moss over all sites and species, and their Spearman rank correlation (*Rho*) and linear relationship with chlorophyll concentrations. Reflectance signals are from key wavelengths over the spectra measured. Regressions were done on logarithmically transformed data where appropriate. Levels of significance are *P < 0.10, ** P <0.05, *** P <0.001.

Reflectance Parameter	*Rho*	Regression	r^2
R280	0.087	NS	
R320	0.057	NS	
R476	0.044	NS	
R526	0.298	LogR526 = -1.72 + 0.000193 x TChl	0.060**
R550	0.362	LogR550 = -1.60 +0.000242 x TChl	0.080***
R680	-0.168	NS	
R850	0.486	R850 = 0.261 + 0.000134 x TChl	0.166***
R850-280	0.484	R850-280 = 0.254 + 0.000133 x TChl	0.161***
R850-320	0.482	R850-320 = 0.253 + 0.000133 x TChl	0.163***
R850-480	0.485	R850-480 = 0.251 + 0.000134 x TChl	0.166***
R850-526	0.475	R850-526 = 0.239 + 0.000125 x TChl	0.161***
R850-550	0.483	R850-550 = 0.259 + 0.000125 x TChl	0.171***
R850-680	0.488	R850-680 = 0.239 + 0.000132 x TChl	0.179***
R850/480	0.292	R850/480 = 31.83 + 0.0140 x TChl	0.050**
R850/526	0.063	NS	
R850/550	-0.007	NS	
R850/680	0.462	R850/550 = 13.46 + 0.0139 x TChl	0.107***
R680/850	-0.498	R680/850 = 0.103 + 0.00000651 x TChl	0.122***
$_{\lambda RE}$	0.335	$R_{\lambda RE}$ = 698.8 + 0.00139 x TChl	0.069**
$_{RE}$	0.488	R_{RE} = 0.00562 + 0.00000365 x TChl	0.147***
PRI	0.273	PRI = -0.194 + 0.00000596 x TChl	0.059**
NDVI	0.498	NDVI = 0.813 + 0.000113 x TChl	0.125***
EGFR	0.196	NS	
EGFN	0.107	NS	
NPCI	-0.146	NS	
CHB	0.408	CHB = 0.198 + 0.000162 x TChl	0.151***

Table 7. Reflectance of the moss *Grimmia antarctici* when wet and dry. Samples are from ridges (*N*=4). Tests are paired t-tests for samples that were dry and then rehydrated for 5 minutes. NS signifies a non-significant difference at the *P*=0.10 level).

	Dry	Wet	*t*	*P*
R320	0.0093 ± 0.0014	0.0104 ± 0.0013	NS	
R526	0.026 ± 0.004	0.0022 ± 0.004	-2.62	0.079
R550	0.031 ± 0.010	0.037 ± 0.004	NS	
R850	0.302 ± 0.020	0.294 ± 0.021	NS	
$_{RE}$	0.0052 ± 0.0001	0.109 ± 0.005	-21.12	0.0002
$_{\lambda RE}$	713.25 ± 0.75	699.9 ± 0.41	15.63	0.006
PRI	-0.146 ± 0.042	-0.118 ± 0.046	-4.87	0.016
NDVI	0.881 ± 0.020	0.890 ± 0.018	NS	
CHB	0.287 ± 0.021	0.260 ± 0.031	2.92	0.062

Table 8. Summary of effects of reflectance parameters and pigments over species, sites and microtopography. X indicates a main effect of the factor at *P*<0.10, XX at *P*<0.05 and XXX at *P*<0.001. N/A signifies not applicable because measurements were not made.

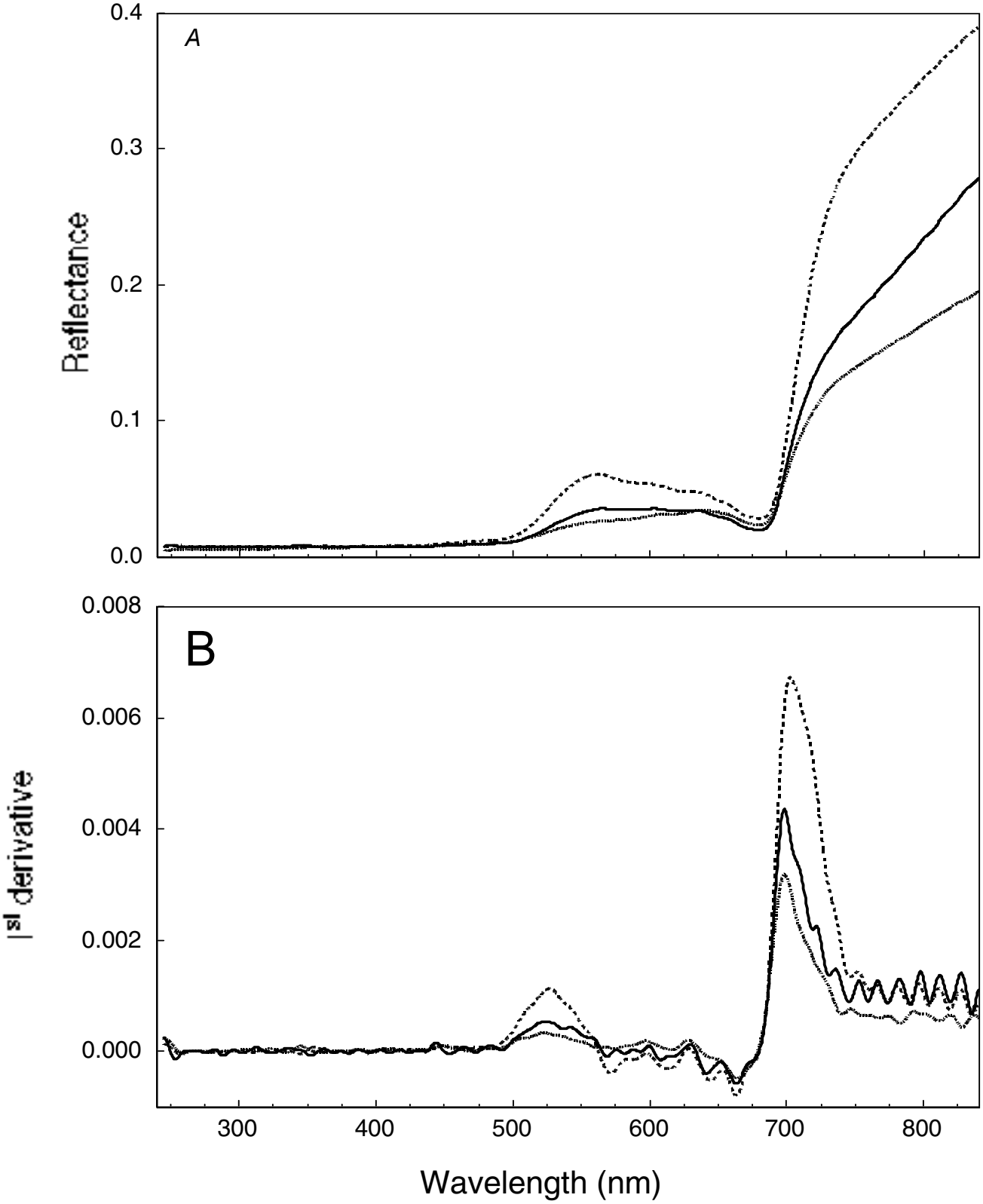
Parameter	Species	Site	Microtopography	Water Content
Reflectance				
R320				
R526	XXX	XX		X
R550	XX	XX		
R850	XX	XX		
$_{RE}$	XXX	XX		XXX
$_{\lambda RE}$	XX	XXX		XX
PRI			XXX	XX
NDVI				
NPCI				
EGFR		XX		
EGFN		XX		
CHB	X	XX		X
Pigments				
Anthocyanins $A_{526\ diff}g^{-1}$ fwt				N/A
Anthocyanins/TChl. $A_{526\ diff}mol^{-1}$	XX			N/A
UV absorbing pigments Mean $A_{(320\text{-}280nm)}$ g^{-1} dwt	XXX	XX	N/A	N/A
Total Chlorophyll nmol g^{-1} fwt	X		XXX	N/A
Chl a:b ratio				N/A
VAZ/TChl mmol mol^{-1}	XX	XX	XX	N/A
%(AZ/VAZ)	XX	XX	XX	N/A
Tcar./Tchl. mmol mol^{-1}	X	XX	XX	N/A

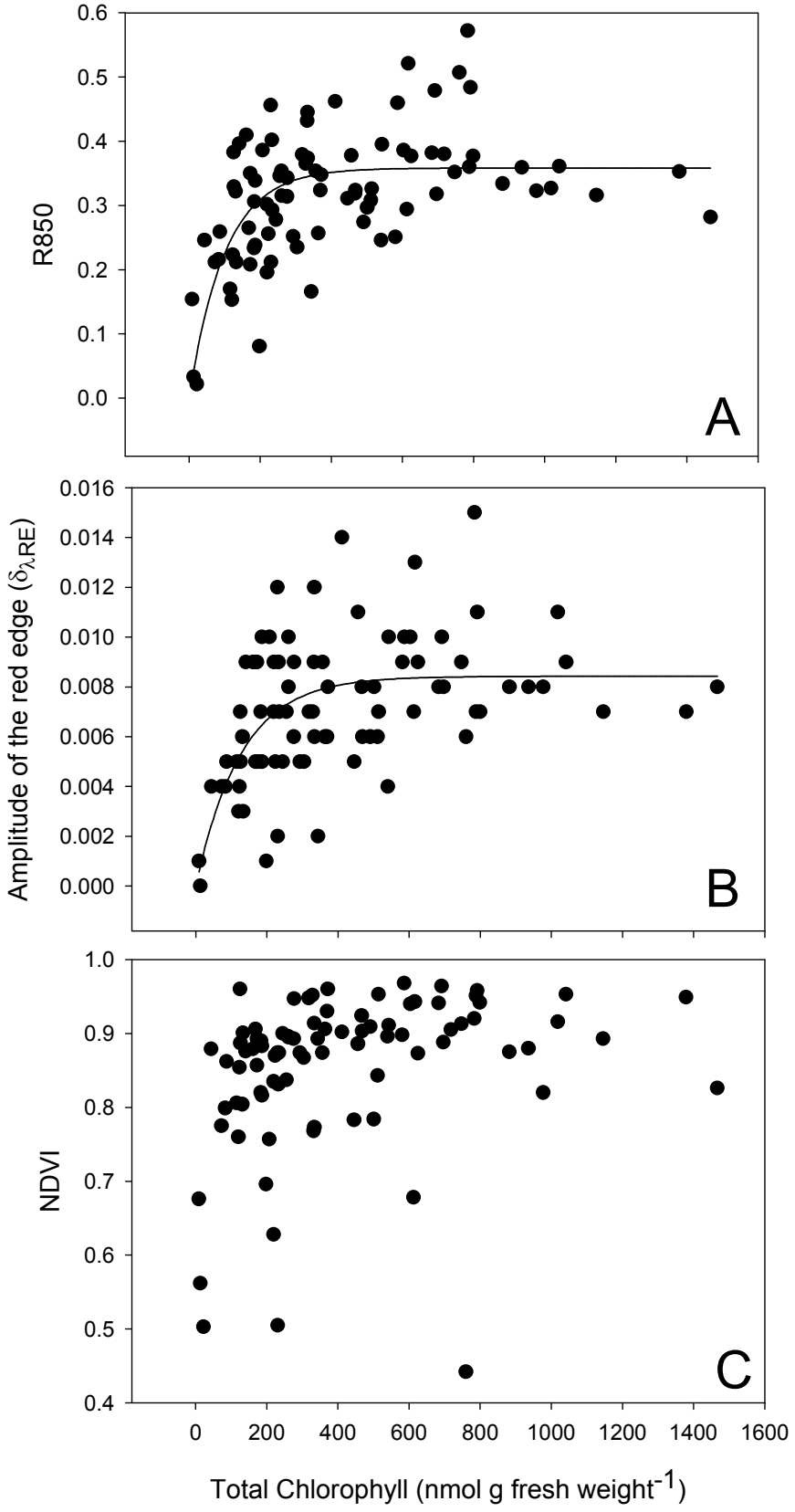
Figure Captions

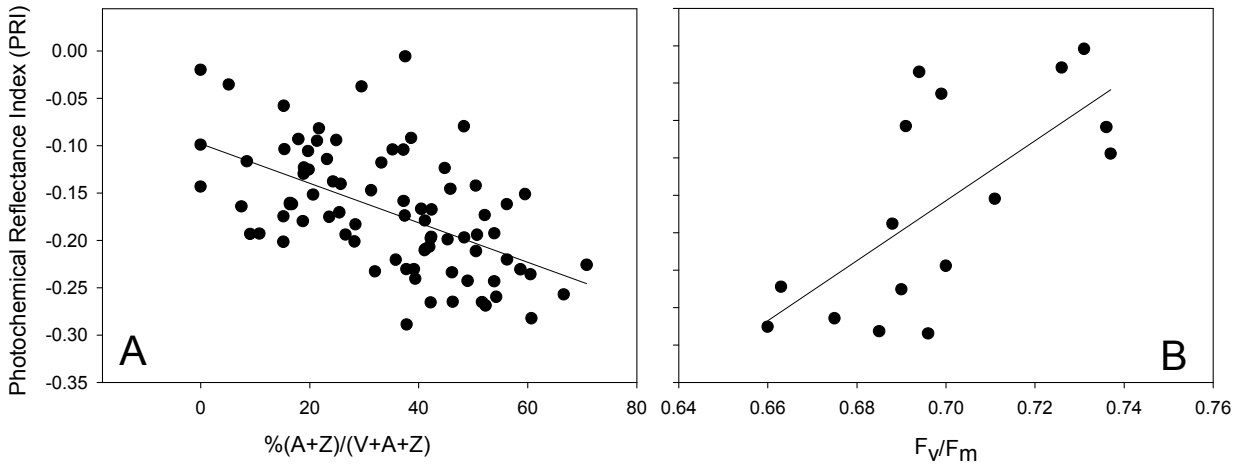
Figure 1. Mean reflectance spectra (A) and first derivative (B) of all samples of the three common Antarctic moss species: *Bryum pseudotriquetrum* (dotted line, N=3), *Ceratodon purpureus* (dashed line, N=5) and *Grimmia antarctici* (solid line, N=25) collected during the 1997/8 season from the Robinson Ridge and Red shed sites.

Figure 2. Relationships between total chlorophyll concentration of moss and the reflectance at 850 nm (A), amplitude of the red edge, λ_{RE} (B), and the NDVI (C). Lines of best fit are exponential curves: R850 = 0.358 + [1-exp(-0.0099 x TChl)], r^2 = 0.352; λ_{RE} = 0.0084 + [1-exp(-0.0079 x TChl)], r^2 = 0.308.

Figure 3. Relationships between the Photochemical Reflectance Index (PRI) and the proportion of the xanthophyll-cycle pigments in their active form (%A+Z/V+A+Z) (A) and the chlorophyll fluorescence parameter Fv/Fm (B). Regression equations are: PRI = -0.0976 + (-0.00209 x %AZ/VAZ), r^2 = 0.298; and PRI = -1.285 + (1.604 x F_v/F_m), r^2 = 0.427.


A
B
Reflectance
|sl derivative
0.4
0.3
0.2
0.1
0.0
0.008
0.006
0.004
0.002
0.000
300
400
500
600
700
800
Wavelength (nm)


R850
0.6
0.5
0.4
0.3
0.2
0.1
0.0
A
Amplitude of the red edge ($\delta_{\lambda RE}$)
0.016
0.014
0.012
0.010
0.008
0.006
0.004
0.002
0.000
B
NDVI
1.0
0.9
0.8
0.7
0.6
0.5
0.4
C
0
200
400
600
800
1000
1200
1400
1600
Total Chlorophyll (nmol g fresh weight^{-1})


Photochemical Reflectance Index (PRI)
0.00
-0.05
-0.10
-0.15
-0.20
-0.25
-0.30
-0.35
A
0
20
40
60
80
%(A+Z)/(V+A+Z)
B
0.64
0.66
0.68
0.70
0.72
0.74
0.76
Fv/Fm