# PHOTOSYNTHETIC CO$_2$ EXCHANGE AND SPECTRAL VEGETATION INDICES OF BOREAL MOSSES

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# DEDICATION

To Sara – you have wholeheartedly offered your life to the most important career even though you receive little recognition.

# ABSTRACT

Moss dominated ecosystems are an important part of the global terrestrial carbon cycle. Over large areas, remote sensing can be useful to provide an improved understanding of these ecosystems. Two boreal mosses (*Pleurozium* and *Sphagnum*) were assessed using remote sensing based spectral vegetation indices for estimating biochemical capacity and photosynthetic efficiency by varying net photosynthesis rate via changes in water content. In the laboratory, changes in the normalized difference vegetation index (NDVI) and chlorophyll index coincided with declining photosynthetic capacity due to desiccation. This effect was more dramatic in *Sphagnum*. The photochemical reflectance index (PRI) did not vary with changes in $CO_2$ supply as anticipated, possibly due to overriding effects of changing water content. The water band index (WBI) was strongly related to water content but this relationship showed an uncoupling in the field. Bi-directional reflectance measurements indicated that WBI was sensitive to sensor, sun, and moss surface slope angles.

## ACKNOWLEDGEMENTS

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# 1 INTRODUCTION

According to the intergovernmental panel on climate change (IPCC 2001), recent observations of global environmental changes such as elevated atmospheric $CO_2$ concentration, land use change, nitrogen deposition, and climate change are a concern for the functioning of terrestrial ecosystems. The IPCC (2001) reported that global atmospheric $CO_2$ concentration has risen 31% (from approximately 280 ppm to 368 ppm) over the last 200 years largely due to burning of fossil fuels. Associated with the increased levels of greenhouse gases, global mean temperature has already risen $0.6^\circ C$ in the past century (IPCC 2001). These temperatures threaten to continue rising depending on the feedback response of Earth's energy budget.

Potential impacts of such changes on the global terrestrial carbon cycle have motivated the beginnings of global climate change management strategies including the Kyoto Protocol initiated by the United Nations Framework Convention on Climate Change (2005). Alternative strategies are also being proposed such as equity first systems that seek to limit green house gas emissions on a per-person basis (Tonn 2003).

The validity of these initiatives can be supported using projected damage cost estimates of climate change, however these estimates still have large degrees of uncertainty due to unknowns in the projected extent of global climate change (Tol 2005). This uncertainty is due, in part, to our incomplete understanding of how global environmental changes alter photosynthesis and respiration in various ecosystems and how these alterations, in turn, feedback to the climate system (Belyea and Malmer 2004).

Large quantities of carbon move into and out of natural ecosystems (Chapin et al. 2002). Simply described, carbon dioxide is taken up from the atmosphere as a result of photosynthesis and released as a product of the respiration of living cells (Chapin et al. 2002). Methane ($CH_4$) is also released to the atmosphere as a result of anaerobic decomposition (Chapin et al. 2002). Carbon dioxide and methane are two of several gases classed as greenhouse gases because they trap long wave radiation in the atmosphere resulting in higher atmospheric temperatures (Ahrens 2000). Factors such as climate and human disturbance influence ecosystem function and ultimately, the balance between carbon pool size in the biosphere and atmosphere (Chapin et al. 2002). Whether carbon exchange of the biosphere will respond to increased temperature and atmospheric $CO_2$ concentration by net gains (negative feedback) or net losses (positive feedback) of carbon is not well understood, however, various ecosystems will likely respond differently (Chapin et al. 2002).

Of major terrestrial ecosystems, peatlands store nearly one third (about 450 petagrams) of the world's soil carbon pool and this large store of carbon is highly susceptible to being released to the atmosphere (Gorham 1991, Turunen et al. 2002). Peatlands are typically moss or sedge dominated wetland regions with organic soils > 40 cm deep (Hilbert et al. 2000). Despite low $CO_2$ uptake rates relative to many ecosystems, they form due to low decomposition rates and remain net carbon sinks unless the water table and ground surface depart sufficiently (Gorham 1991). Variations in peatland water table depth can occur through changes in the regional hydrologic cycle, energy budgets, or direct human disturbance (Gorham 1991, Hilbert et al. 2000, Frolking et al. 2002).

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Understanding peatland $CO_2$ exchanges in relation to these variables is essential for accurate terrestrial carbon cycle modelling and prediction (Frolking et al. 2002). Knowledge of the response of peatland ecosystems to global environmental change can support management decisions to reduce, or avoid, the release of large net carbon fluxes to the atmosphere, thereby reducing further exacerbation of global environmental change.

The Fluxnet-Canada Research Network (FCRN), part of a larger global flux network, seeks to measure and describe how variation in net ecosystem exchange (NEE) of $CO_2$ in Canadian forests and peatlands interacts with global climate change and human disturbance. One of FCRN's roles is to conduct fundamental applied scientific research on these ecosystems to assist Canadian policy development for promoting long term environmental stewardship. This will influence ecosystem management, greenhouse gas emissions, and fulfilment of the objectives of the Kyoto Protocol and more advanced programs in the future. Fluxnet-Canada has undertaken several approaches to study carbon budgets of forest and peatland ecosystems at various spatial scales. These include leaf and canopy scale carbon flux data acquisition and analysis techniques including tower-based eddy covariance measurements complemented with reflectance measurements from ground, tower, and high altitude (aircraft and satellite) sensors.

Development of remote detection techniques for studying factors that influence NEE have often been intended to enhance extrapolation of localized models of $CO_2$ exchange over large land areas. This 'scaling-up' rationale is commonly noted in vegetative reflectance studies in the field of remote sensing (Gamon et al. 1992, Sellers et al. 1992, Hall et al. 1996, Rahman et al. 2001, and Stylinski et al. 2002). Development of these techniques also enables the use of spectroradiometers as efficient tools for ground-

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based acquisition of biologically relevant reflectance data using portable and tower based sensors (Gamon et al. 1990, Houston 2004). Ground-based reflectance measurements, in combination with other information gathering techniques used by FCRN, can be used to enhance understanding of the response of vegetation to environmental changes and improve ecosystem models (Frolking et al. 2002). Understanding the dynamic linkages between vegetation spectral response and leaf characteristics related to $CO_2$ sequestration would improve interpretation of ecosystem response to environmental conditions. This information can then be used for two interrelated applications. Firstly, to improve ecosystem $CO_2$ exchange models to reduce uncertainty in environmental change forecasts. Secondly, to provide scientific support for decisions relating to land use and greenhouse gas emissions.

This thesis presents an investigation of how the spectral reflectance of forest and peatland mosses respond to limitations to $CO_2$ uptake. Limitations to $CO_2$ uptake can be divided into two classes: $CO_2$ supply, and the biochemical capacity to incorporate $CO_2$. For mosses, these two classes of limitations have been studied by varying water content (Williams and Flanagan 1996). The investigation of spectral vegetation indices is based upon this model system as shown in Figure 1. Figure 1 is briefly summarized in the remainder of this paragraph and in more detail in the following sections. Figure 1 shows how maximum net $CO_2$ uptake (1) occurs at mid-range water content. Reductions in net $CO_2$ uptake result from reduced biochemical capacity related to desiccation (2) and reduced $CO_2$ supply caused by a thickened water boundary layer (3). The NDVI and CI should respond similarly to each other (B) because they both compare visible to near infrared reflectance. The NDVI and CI should change in response to chlorophyll

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absorption that indicates biochemical capacity. PRI is anticipated to indicate dynamics in zeaxanthin concentration related to fluctuations in the amount of photons in excess of that usable for photochemistry (C). At high water content, $CO_2$ is limiting to photosynthesis and excess photons must be quenched via zeaxanthin to prevent damage to chlorophyll.

Mosses do not have stomata and therefore, $CO_2$ supply is primarily influenced by large passive variations in moss water content (Clymo and Hayward 1982) (Figure 1A). This short-term dry-down response of moss shows how net $CO_2$ uptake is reduced at low water contents due to declining biochemical capacity. Low water contents result in low net photosynthetic uptake because cell activity and metabolism declines (Silvola 1990, Schipperges and Rydin 1998, Proctor 2000). At very high water contents, net $CO_2$ uptake is suppressed as $CO_2$ supply to the chloroplasts is impeded since $CO_2$ diffuses slowly through water (Silvola 1990). These principles provide a framework to evaluate short-term changes in moss spectral reflectance under controlled conditions. Measurement of leaf (or moss) reflectance offers a tool to help explain some of the mechanistic reasons for changes in net $CO_2$ uptake.
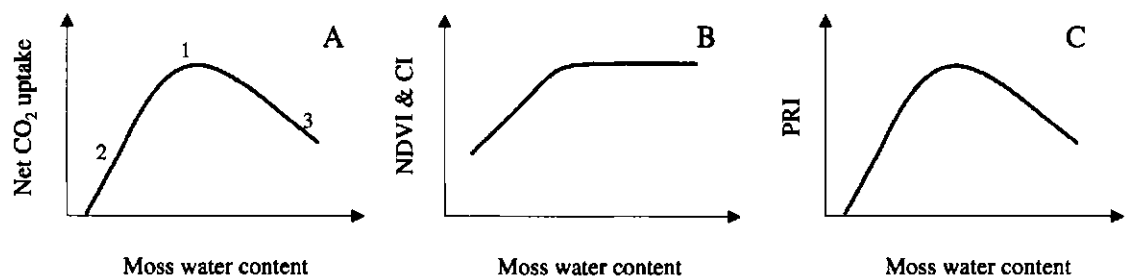
Figure 1: Conceptual diagrams of the theoretical response of net photosynthesis (A), NDVI and CI (B), and PRI (C) to short-term dry down of mosses. Axis scales are relative linear hypothetical approximations. Details pertaining to this presentation are contained in the text.

## 1.1 Spectral vegetation indices

Spectral reflectance is the ratio of reflected to incident electromagnetic radiation flux assuming no change in frequency (Nicodemus et al. 1977). Reflectance is independent of illumination intensity and is an inherent property of an object (Peddle et al. 2001a). Changes in passive optical (visible and near infrared) reflectance of vegetation occur with changes in pigment content, water content, and cell structure as described by Gausman (1985) and described in more recent works to be reviewed in this chapter. Spectral vegetation indices are simple mathematical ratios of reflectance wavebands commonly used to provide relative estimates of biological information (Rouse et al. 1974, Chen 1996). Three general styles of vegetation indices include simple ratio, complex ratio, and derivative indices. Simple ratio indices divide the reflectance of one band by another and therefore are simply a measure of the slope between two points on a spectral

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response curve. Complex ratios are elaborations upon the simple ratio to either account for other bandwidths or to normalize one band versus another. Normalization enables a linear response of the index to changes in reflectance and will remain constant if there are proportionate changes in reflectance for the two bandwidths selected. Derivative indices provide information about changes in slope for an area of interest on the spectrum.

Spectral vegetation indices have often been used to estimate green biomass relating to leaf area index (LAI) and ecosystem net primary production (NPP) (Rouse et al. 1973, Chen 1996). Vegetation indices have also correlated with net $CO_2$ exchange in boreal forest and peatland ecosystems (Nichol et al. 2000), agricultural systems (Penuelas and Inoue 2000), and evergreen chaparral shrub ecosystems (Stylinski et al. 2002). Since net photosynthesis rate is dependent upon many factors, these studies found it useful to compare the response of more than one vegetation index while net photosynthesis rate varied. Previous studies have compared reflectance of mosses with water content (Fernandes et al. 1996, Bubier et al. 1997, Lovelock and Robinson 2002, Houston 2004) but have either not explored vegetation indices or did not evaluate indices in controlled laboratory conditions with a wide range of water content values. Accordingly, the approach taken in this thesis utilizes spectral vegetation indices as a tool to help explain why certain observed changes in moss net $CO_2$ exchange occur under given environmental conditions. A rigorous experimental design involving the comparison of a variety of key vegetation indices has therefore been developed in this research using a controlled laboratory setting with relevant concepts also extended to the field. The following subsections provide descriptions of selected vegetation indices (Table 1) and a rationale for using them in this study.

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Table 1: Spectral vegetation indices used in this study

| Spectral vegetation index | Definition ($R_\#$ = reflectance of 1 nanometer bandwidth centered on #) | Source |
|---|---|---|
| Normalized difference vegetation index | $NDVI = \dfrac{(R_{800} - R_{670})}{(R_{800} + R_{670})}$ | Houston 2004, Rahman et al. 2001 |
| Chlorophyll index | $CI = \dfrac{(R_{750} - R_{705})}{(R_{750} + R_{705})}$ | Gitelson and Merzlyak 1994 |
| Photochemical reflectance index | $PRI = \dfrac{(R_{531} - R_{570})}{(R_{531} + R_{570})}$ | Gamon et al. 1993, Gamon et al. 1997 |
| Water band index | $WBI = \dfrac{R_{900}}{R_{970}}$ | Sims and Gamon 2003, Houston 2004 |

1.1.1 Normalized difference vegetation index and chlorophyll index

The normalized difference vegetation index (NDVI) is a ratio of visible to near infrared (NIR) reflectance (Table 1) (Rouse et al. 1973). It is a value commonly calculated from air and space borne sensors and it is correlated with ecosystem leaf area index (LAI), biomass, and productivity (Chen 1996). Normalization refers to the calculation of the difference in reflectance between the two wavebands divided by their sum. This calculation can reduce the effects of radiometric errors caused by variable

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illumination angle and atmospheric attenuation and emphasizes the relative offset

between the two bands (Rouse et al. 1973). Vegetation indices were originally developed

for broadband multispectral satellite sensors (Rouse et al. 1973) but very narrow

wavebands are commonly substituted when spectroradiometer spectral resolution permits

(Rahman et al. 2001, Lovelock and Robinson 2002, Stylinski et al. 2002). Mature,

healthy, green vegetation yields high NDVI values because visible radiation (e.g. 670

nanometers) is strongly absorbed by chlorophyll and NIR radiation (e.g. 800 nm) is

highly reflected due to high intercellular airspace enabling many routes for NIR

refraction through cell walls of spongy mesophyll (Gausman 1985). High NDVI is

therefore indicative of high photosynthetic capacity and has been expressed as an

estimator of the fraction of absorbed photosynthetically active radiation (fAPAR)

(Myneni and Williams 1994). This is, the fraction of photosynthetic photon flux density

(PPFD) absorbed by the leaf relative to the proportion reflected by and transmitted

through the leaf (Myneni and Williams 1994).

Based on previous studies of moss photosynthetic capacity, Figure 1B illustrates

how NDVI is likely to respond to short-term changes in moss water content. At high

water content, NDVI should remain unchanged since biochemical capacity (chlorophyll

content) is expected to remain unaffected by excess water in the short term (Williams and

Flanagan 1998). As water content declines below the optimum for net $CO_2$ uptake, NDVI

is expected to decline due to reduced absorption by chlorophyll (Schipperges and Ridden

1998). The chlorophyll index (CI) (Table 1) is a modified form of NDVI used for

estimating chlorophyll content (Gitelson and Merzlyak 1994, Gitelson et al. 1996, Gamon

and Surfus 1999). Chlorophyll index uses the red edge, or the extent of increase in

reflectance of radiation between the red (705 nm) and NIR (750 nm) portions of the

electromagnetic spectrum (Gitelson and Merzlyak 1994). The advantage of using the

chlorophyll index in conjunction with NDVI is that the 705 nm band used for CI is less

susceptible to saturation at high chlorophyll contents than the 670 nm band used for

NDVI (Gitelson and Merzlyak 1994, Datt 1998).


1.1.2 Xanthophyll pigments and the photochemical reflectance index

The photochemical reflectance index (PRI) (Table 1) has been related to

photosynthetic light use efficiency (LUE) for a wide range of vascular plant species

(Gamon et al. 1997, Penuelas et al. 1997). This is based on the demonstration of changes

in reflectance at approximately 531 nm in association with changes in a set of carotenoid

pigments known as xanthophylls (Gamon et al. 1992, 1993). This section firstly describes

the function of xanthophyll pigments and factors that affect their concentrations.

Secondly, it describes their influence on leaf reflectance and the hypothesized effects of

moss water content on PRI.


1.1.2.1 Xanthophyll physiology

Xanthophyll pigments have two main functions as described in many studies

including those by Demmig et al. (1988), Demmig-Adams and Adams (1996a), Lambers

et al. (1998), Blankenship (2001), Adams (2004), and Holt et al. (2005). In a form called

violaxanthin ($V$), they assist chlorophyll to acquire solar energy for photosynthesis. Due

to environmental variability, the supply of photon energy almost never exactly meets the

ability of the plant to use this energy. If PPFD threatens to exceed the maximum amount

of photon energy that can be used by photosynthesis, $V$ rapidly converts to zeaxanthin

($Z$). Zeaxanthin protects the plant by harmlessly dissipating excess excitation energy as

heat (Demmig-Adams and Adams 1996a). If it were not for this dissipation, chlorophyll,

specifically proteins of the photosystem II (PSII) reaction centers within the chloroplasts,

would be damaged (Lambers et al. 1998). During periods when PPFD falls below the

maximum intensity that can be used by photosynthesis, $Z$ can rapidly convert back to $V$.

As a result of this dual role, these two forms of xanthophyll vary in concentration relative

to incident PPFD and the leaf's capacity to use this irradiance (Demmig-Adams and

Adams 1996a). If the xanthophyll cycle is ineffective, extensive damage caused by

excess excitation energy can lead to chlorophyll oxidation or bleaching that can cause the

leaves to appear yellowed (Lambers et al. 1998). This has been termed photodamage

(Lambers et al. 1998) and results in decreased light use efficiency of the plant for many

days until damaged proteins can be replaced (Taiz and Zeiger 2002). Photodamage

should not be confused with photoinhibition. Photoinhibition is the decrease in

photosynthetic efficiency correlated with rises in $Z$ concentrations caused by excess

PPFD (Adams et al. 2004).

The magnitude, timing, and recovery of xanthophyll interconversion appear to

vary with plant species, acclimation, and the temporal characteristics of environmental

fluctuations. For example, $Z$ levels in leaves acclimated to high light (sun leaves) closely

track diurnal PPFD variability while shade-acclimated leaves maintain relatively high

concentrations of $Z$ for longer periods of time following sunfleck exposure (Lambers et

al. 1998, Taiz and Zeiger 2002). In a series of experiments by Demmig Adams et al.

(1998), following the exposure of shade leaves to 24 hours of high light (1200 $\mu$mol m$^{-2}$

$s^{-1}$), 24 hours was required for $Z$ concentrations to fall significantly during recovery in low light (10 μmol $m^{-2}$ $s^{-1}$). They also observed that exposure of shade leaves to high light for only 3 hours followed by a return to low light yielded a much more rapid drop in $Z$. For these shade leaves, xanthophyll concentrations relative to total carotenoids rose from 10% to 20% during a 72-hour recovery time at 10 μmol $m^{-2}$ $s^{-1}$ PPFD. In contrast to this, leaves acclimated to 1200 μmol $m^{-2}$ $s^{-1}$ PPFD showed a drop in xanthophyll concentrations from about 30% to 27% during recovery (Demmig-Adams et al. 1998). Over the longer term, leaves acclimated to high light contain a larger concentration of xanthophylls relative to total carotenoids (Demmig-Adams 1998) and larger concentrations of carotenoids relative to chlorophyll (Lambers et al. 1998). Results from Demmig et al. (1988) showed that water stress of a hardy evergreen shrub slowed recovery of $V$ from $Z$. These shrubs were also capable of increasing their total $Z$ pool under excess PPFD not only due to interconversion of $V$ but also due to their ability to quickly manufacture (within days) additional $Z$ from β-carotene (Demmig et al. 1988). Some species, such as *Populus balsamifera* (Balsam poplar), do not appear capable of increasing their xanthophyll pool in this way and are likely more susceptible to photodamage during long periods of exposure to high PPFD (Demmig et al. 1988). These examples illustrate that xanthophyll interconversions and pool sizes vary with environmental conditions and, as described below, influence the reflectance of the plant.

1.1.2.2 Leaf reflectance and the photochemical reflectance index

        Spectral response of plants is characterized by high reflectance and transmittance in the near infrared portion of the electromagnetic spectrum and high absorption of visible radiation, particularly blue and red light (Gausman 1985). The chlorophylls are

the primary cause of this visible light absorption while carotenoids also contribute to absorption particularly between 400 and 500 nm (Taiz and Zeiger 2002). Bilger et al. (1989) found that absorbance changes centred at 505 nm were related to $V$ conversion to $Z$ when *Gossypium hirsutum* (cotton) leaves, adjusted to low light (100 μmol m$^{-2}$ s$^{-1}$) were exposed to high intensity light (1300+ μmol m$^{-2}$ s$^{-1}$). Within 4 minutes of exposure, Bilger et al. (1989) found a 30 to 50% increase in $Z$ relative to the total xanthophyll pool and an increase in reflectance at 505 nm of between 3 and 5 % of incident radiation. The effects on leaf spectral reflectance resulting from xanthophyll conversions were explored further by Gamon et al. (1990) using spectroradiometer measurements of *Helianthus annus* (sunflower). Correlations were found between changes in reflectance centered at 531 nm and $Z$ concentrations (Gamon et al. 1990). Further investigation of the 531 nm waveband in relation to a wide variety of angiosperm species revealed consistency with original findings although the peak reflectance change varied with species between 517 nm for *Crassula argentea* (jade plant) and 539 nm for *Rhus ovata* (sugar sumac) (Gamon et al. 1993). The physiological reflectance index, later renamed the photochemical reflectance index (PRI), was proposed by Gamon et al. (1992) and involved the measurement of reflectance at 531 nm normalized to 570 nm. Reflectance at 570 nm was selected to normalize for leaf characteristics such as chloroplast movement. Chloroplast movement is a response to high intensity irradiance below 500 nm particularly in shade-tolerant species (Lambers et al. 1998). Under these circumstances, chloroplasts align themselves parallel to incident radiation to avoid excess absorption (Lambers et al. 1998). The empirical support provided by Demmig-Adams et al. (1996b) for the relationship between PSII efficiency and $Z$ levels supported the basis for using measurements of $Z$

concentrations to estimate light use efficiency. Significant relationships were found

between leaf level measurements of PRI and variability in photosynthetic radiation use

efficiency (PRUE) for a wide range of species (Gamon et al. 1997, Penuelas et al. 1997).

High $Z$ concentration associated with excess light was found to decrease reflectance at

531 nm relative to reflectance at 570 nm and therefore yield a low (more negative) PRI

value. Stylinski et al. (2002) found positive relationships ($r^2 > 0.6$) between PRI of

chaparral shrubs and net $CO_2$ uptake of leaves throughout the growing season. As

mentioned above, the photochemical reflectance index (PRI) (Table 1) has been related to

photosynthetic light use efficiency (LUE) for a wide range of vascular plant species

(Gamon et al. 1997, Penuelas et al. 1997).

Based on these studies, Figure 1C shows how PRI for mosses is anticipated to

correspond with variation in net photosynthesis caused by shifts in moss water content.

As water content is increased above optimal levels for net photosynthesis, $CO_2$ supply

should become limiting resulting in reduced demand for photon-derived energy. To

safely dissipate excess excitation energy as heat, greater quantities of $V$ would be

converted to $Z$. This should result in increased absorbance of radiation centred at 531 nm

thereby causing reductions in PRI. Likewise with water contents below optimal for net

photosynthesis, $Z$ concentrations are hypothesized to rise to assist the protection of

chlorophyll from damage due to radiation stress. Monitoring NDVI and CI in conjunction

with PRI enables assessment of the stability of chlorophyll concentrations and

intercellular structure as a basis to interpret PRI and provide information about the

effectiveness of this protection against photodamage.

1.1.3 Water band index

It may be possible to calculate moss net photosynthesis rate by estimating moss

water content using the water band index (WBI) (Table 1). Models of moss

photosynthesis exist with mechanistic controls by illumination intensity, temperature,

atmospheric $CO_2$ concentration, and water content (Williams and Flanagan 1998). Of

these controls, water content is often the variable that cannot be easily measured in the

field. Reflectance data could provide water content values as a model input based on the

dynamic influence of water content on the absorption of radiation. To estimate water

content from spectral data, middle infrared bands are commonly used; although a strong

absorption bandwidth related to leaf water content also exists in the near infrared centred

at 970 nm (Penuelas et al. 1993). Penuelas et al. (1993) noted that observed reflectance at

970 nm also seemed to be influenced by slowly reversible changes in internal cell

structure and cell wall elasticity related to drying of vascular plant leaves. Sims and

Gamon (2003) defined a water index and showed that it was strongly related to water

content in various species. Field spectral measurements may provide an efficient way to

derive moss water content for the calculation of net $CO_2$ exchange using models of moss

photosynthesis.

1.1.4 Spectral vegetation indices: advantages and limitations

For many applications, spectral vegetation indices have advantages such as the

potential to estimate or derive vegetative characteristics (e.g.: LAI), they are generally

computationally simple, they can partially compensate for measurement variability

induced by atmosphere and instrumentation, and they can produce ranges of values

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broadly interpretable by users from various specializations (Crippen 1990). Vegetation indices are disadvantaged by the fact that they are not always appropriate for accurately modeling plant and ecosystem characteristics since they only incorporate a small number of the available spectral bands (typically only two) yet many others are available, particularly with hyperspectral image data. Another disadvantage is that vegetation indices are intended to obtain information on vegetation yet are based on pixels that include information from both vegetated and non-vegetated sources in their field of view. These include soil and background brightness, species intermixture, shadowing, and canopy structure (Baret and Guyot 1991). In the latter case, vegetation indices have no explicit mechanism to account for canopy geometry or plant structure, factors that affect spectral response and can drive vegetation information extraction. Approaches such as spectral mixture analysis (SMA) and canopy geometric optical reflectance models address many of these disadvantages and have been shown to provide improved information content than vegetation indices (Hall et al. 1996, Peddle et al. 1999, Peddle et al. 2001b). Another problem with vegetation indices is that they can be prone to level off, or saturate, as biophysical parameters (e.g.: LAI) increase in value past a certain point (Haboudane 2004).

In addition to this, limitations in spectral and spatial resolutions can also hinder the interpretation of biological information from reflectance data. High spectral resolution (i.e.: ~10 nm) is particularly important to make use of indices such as PRI that require this hyperspectral capability to examine very specific portions of the spectrum. Hyperspectral remote sensing systems such as the Airborne Visible Infrared Imaging Spectrometer (AVIRIS) provide the opportunity to calculate indices such as PRI from

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high altitudes. Increased altitude usually results in larger pixel sizes, which in turn reduce

the probability of having pixels with only one end member of interest. Satellite spatial

resolutions are improving to 1 m, which might allow for a view of individual species

although with coarser resolution it is possible that differences in indices such as PRI, may

'average' out especially if investigating ecosystem seasonal change. Investigation of the

effects of decreased spatial and spectral resolution on indices such as PRI might be

effectively conducted at first for agricultural systems with minimal end members where

changing the resolution from leaf to field scale could theoretically yield a predictable

difference in the vegetation index.

For acquiring useful biophysical information from reflectance data, alternatives to

vegetation indices may be provided through techniques such as spectral mixture analysis

(SMA) and the inversion of reflectance models. SMA accounts for multiple feature

components in a pixel and involves partitioning reflectance into pre-selected endmembers

(e.g.: sunlit canopy, sunlit background, and shadow) to determine the reflectance

contribution provided by each of these ground features to the total reflectance (Hall et al

1996). As is common for reflectance modeling, when inverting spectral mixture analysis

(spectral unmixing), numerous land cover combinations are possible for the same total

reflectance value. This procedure may require knowledge of the site that might be

attainable strictly from the imagery. This would involve an integrated knowledge of the

response of each part of the spectrum to numerous possible combinations of

biochemistry, structure, and observation conditions that can be provided via radiative

transfer models such as SAIL, 4-Scale, and LIBERTY.

An effective approach to objectively extract biophysical and structural

information such as forest landcover and leaf area index from satellite reflectance data

has been developed by running canopy geometric-optical reflectance models (e.g. 5-

Scale, Li-Strahler GOMS) in multiple forward mode (MFM) (e.g.: Peddle et al. 2004).

Such a technique could be applied to acquire information about moss physiology by

developing a radiative transfer model for mosses and a spectral library of xanthophyll

cycle pigments and other components. If the objective were to derive light use efficiency

(LUE) from above the canopy using a high spectral resolution sensor, the model would

need to be built upon at least a few important components. Firstly, it would need to be

verified that reflectance shifts resulting from xanthophyll at or near 531 nm are readily

detectable in mosses and representative of changes in LUE over a wide range of water

contents, as investigated in this thesis. Secondly, a detailed biochemical radiative transfer

model incorporating xanthophyll cycle pigments and other carotenoids as well as

chlorophyll, variability in leaf structure and perhaps other leaf components would need to

be developed. Thirdly, seasonal and diurnal xanthophyll cycle shifts of various species

viewed within an individual pixel from a canopy scale or further away would need to

vary in predicable fashion. This would need to account for the prospect that, as

environmental conditions change, different species may not exhibit equivalent changes in

leaf level PRI due to their phenology. In conjunction with this, there is also likelihood of

general differences in photosynthetic capacity between species and disproportionate

influence on canopy PRI by some species relative to others when compared on a pigment

content per ground area basis (e.g.: overstorey versus understorey species). As a result,

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canopy PRI may not accurately indicate ecosystem light use efficiency if measured above canopy.

Application of these methods to other types of vegetation (e.g. large crops) would provide an excellent testing ground for similarities and differences in vascular plant response versus the bryophyte response. For example, observations of PRI, NDVI, and WBI could be measured in conjunction with plant characteristics such as photosynthetic $CO_2$ exchange and pigment contents in relation to environmental parameters such as soil moisture. Compared to WBI measurements of mosses, WBI for vascular plants would likely not show as strong of a utility because vascular plants are more capable of regulating leaf water content via vascular tissues and stomata.

## 1.2 Bi-directional Reflectance

The spectral vegetation indices described above offer the potential to provide information about shifts in biochemical capacity (NDVI and CI), photosynthetic efficiency (PRI), and water content (WBI) of mosses. However, many factors have the potential to confound laboratory and field spectroradiometer-derived reflectance as summarized by Milton (1987), Milton and Goetz (1997), and Hatchell (1999). One of these factors is that the reflectance of a surface can vary depending on the position of the viewer and the position of the illumination source. This phenomenon is referred to as bi-directional reflectance since the perceived reflectance of an object depends on the angle of both the viewer and illumination source (Nicodemus et al. 1977). The confounding effects of bi-directional reflectance can be a concern for remote sensing applications and

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advances to account for this have included use of multi-angle viewing capabilities of sensors such as the System Pour L'observation de la Terre (SPOT 2004), the Moderate Resolution Imaging Spectroradiometer (MODIS) (Schaaf et al. 2002; Vanacker et al. 2005), and others. Additionally, if the positions of the viewer and illumination source remain constant but the surface geometry changes for a given target (i.e. slope, aspect), the reflectance observed by the viewer can change. Characterizing the extent of how the surface/subsurface of an object influences the directional reflectance of incident radiation upon it can involve modelling the bi-directional reflectance distribution function (BRDF) of the object (Nicodemus et al. 1977). This continuous hemispherical reflectance pattern of a surface can be interpolated from many reflectance measurements of a single target recorded from many azimuth and zenith angles. A goniometer, further described in section 2.6, can assist in the collection of these measurements by maintaining the sensor foreoptic a constant distance from the target over a full range of sensor view angles and azimuths (Turner 1998, Painter et al. 2003, Coburn and Peddle 2005). The BRDF can than be used as a simulated reflectance 'library' of all possible illumination and viewing angle combinations of a surface (Milton 1987, Turner 1998). Through the examination of bi-directional reflectance effects of moss, this research addresses a potential challenge associated with relating controlled laboratory measurements to the field.


## 1.3 Objectives

If reflectance measurements can be used to provide information about net photosynthesis rates in the laboratory under controlled conditions, it may be possible to use reflectance to derive information about net photosynthesis in ecological experiments

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in the field. Remote sensing techniques provide the advantage of a less invasive, non-destructive, as well as a potentially more efficient approach to acquiring information that may be of particular relevance to benefiting multi-disciplinary ground-based applications alone. In addition, remote sensing provides many options to detect relevant information from high altitude airborne and space platforms. Users of information acquired from these platforms can benefit from an understanding of the how radiation transfer is influenced under various degrees of experimental complexity. For example, remote sensing science as well as other disciplines can simultaneously benefit from research into factors that may complicate reflectance measurements such as bi-directional reflectance distribution function (BRDF), which can be assessed and incorporated. The BRDF acquired from controlled measurements can be further developed in field-based studies to be used as an additional information source in conjunction with vegetation indices and other spectral analysis techniques. Differences in the controlled parameters of otherwise similar experiments can provide a testing ground for remote sensing techniques.

To study the influence of changes in moss water content on net photosynthesis and spectral vegetation indices, experiments were conducted on mosses from the Western Peatland flux station of the Fluxnet-Canada Research Network. Selected spectral vegetation indices (NDVI, CI, PRI, and WBI) and net photosynthesis in boreal mosses (*Pleurozium* and *Sphagnum*) were measured as water content was varied under controlled conditions in the laboratory. The response of these indices to water content was also investigated in the field. Further investigation was conducted in the laboratory to address differences observed between field and laboratory results. This involved examining bi-directional reflectance effects of moss to estimate the impact of reduced control over the

geometry of light source and sensor positions in field measurements (i.e. illumination and view angles).

Completion of these objectives will indirectly contribute to environmental change research by helping to improve remote sensing data interpretation and collection methods. These improvements will enhance the extraction of ecosystem physiological information from reflectance data. Specifically, if these spectral vegetation indices respond as predicted, ground level reflectance measurements in the field could be used to derive photosynthetic capacity (NDVI and CI), photosynthetic efficiency (PRI), and water content (WBI) in boreal mosses. This research also attempted to quantify some of the complications associated with deriving this information from reflectance. This was done with the goal of improving the utility of remote sensing for learning how ecosystems respond to environmental change. A deeper understanding of how ecosystems function can then be used to provide accurate information to help forecast climate change.

# 2 METHODS

## 2.1 Field study site

The Western Peatland flux station (Figure 2) is located approximately 100 km

north east of Athabasca, Alberta and is one of two peatland flux stations in the Fluxnet-

Canada Research Network (the other is the Mer Bleu bog near Ottawa, Ontario). The

Western Peatland main site (54.954°N, 112.467°W, WGS84) is a moderately rich treed

fen that is representative of a large proportion of boreal peatlands in Western Canada. It

has an average peat depth of approximately 2 meters and mean annual precipitation and

air temperature are 507 mm and 2.1°C respectively (Athabasca Weather Station,

Environment Canada). Species associated with this ecosystem include *Picea mariana*

(black spruce), *Larix laricina* (tamarack), *Betula pumila* (bog birch), *Adromeda polifolia*

(bog rosemary), *Ledum groenlandicum* (Labrador tea), *Salix* sp. (willow), *Pleurozium*

*schreberi* (big red stem moss) (Figure 3), and *Sphagnum* (peat mosses) such as *Sphagnum*

*teres* (thin-leafed peat moss) (Figure 3), *Sphagnum fuscum* (rusty peat moss), *Sphagnum*

*magellanicum* (midway peat moss), and *Sphagnum angustifolium* (poor fen peat moss).

*Pleurozium* and other feather mosses are typically associated with more densely treed

portions of the peatland found slightly higher above the water table. *Sphagnum* mosses

are more common at this site and are found in areas with lower tree canopy and a denser

shrub canopy. Aboveground biomass of green mosses comprises just under half (92 g C

$m^{-2}$) of forest understorey aboveground biomass (205 g C $m^{-2}$) and slightly more than one

sixth of total ecosystem aboveground biomass (540 g C $m^{-2}$) (Flanagan unpublished

data). Estimates of net primary productivity (NPP) at the Western Peatland main site

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suggest that mosses contribute approximately two thirds of total ecosystem NPP

(Flanagan unpublished data).
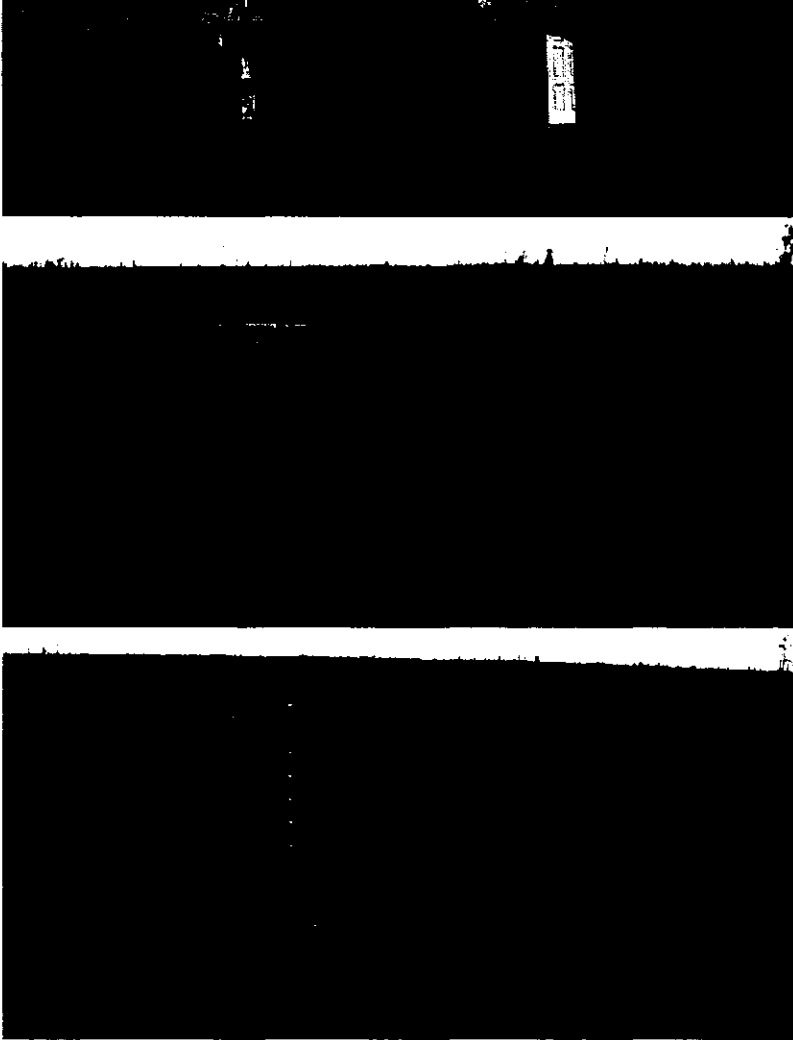
Figure 2: Photographs of the Western Peatland main site summer 2003 (top - by LB Flanagan), August 18 (middle), and October 14, 2004 (bottom). The bottom two photos show an adjacent 6 m net radiometer tower standing above most of the *Larix laricina* and *Picea mariana* treetops.
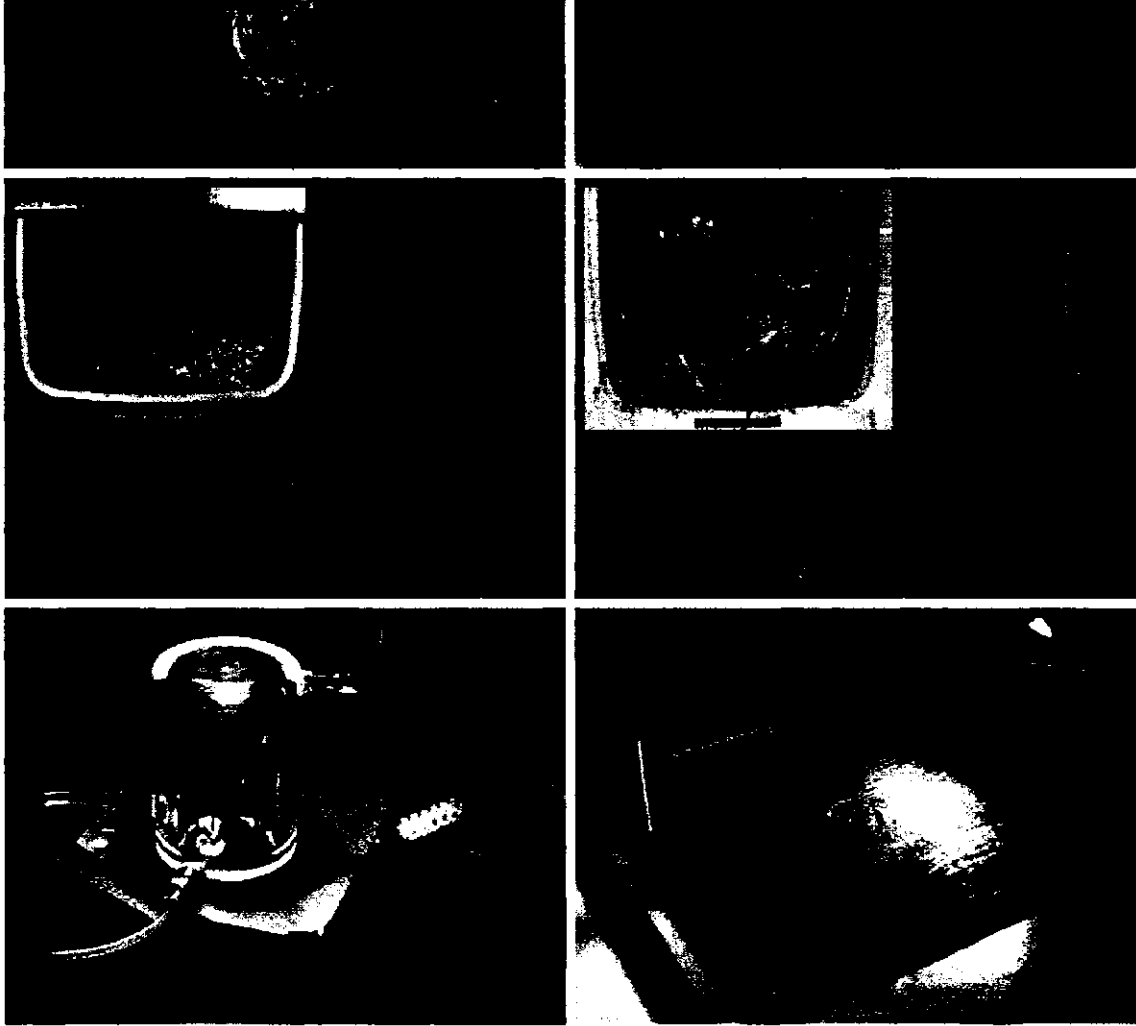
Figure 3: Photographs of various methods and experimental apparatus. *Pleurozium schreberi* (upper left) and *Sphagnum teres* (upper right) being collected using a glass cuvette for water content measurement in the field. *Pleurozium* (middle left) and *Sphagnum* (middle right) maintained in containers in growth chambers and used for laboratory experiments (insets of their containers). Dry down experimentation apparatus: water-jacketed gas exchange chamber for measuring net photosynthesis rate of moss (lower left) and removable stage for holding moss in a cuvette underneath the spectradiometer foreoptics during reflectance measurements (lower right).

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## 2.2 Collection of moss samples

For laboratory experimentation, mosses (*Pleurozium schreberi* and *Sphagnum teres*) were collected from the field during the summer of 2004. Mosses were selected for healthy green appearance and growth characteristics representative of the majority of moss samples observed in the field. Sections of moss 60 cm x 30 cm x 20 cm deep were carefully cut and extracted from the forest floor and then placed into large plastic bins. The collections were comprised of relatively homogenous lawns with respect to moss species and physical appearance (colour, texture, capitula diameter, and density) as shown in Figure 3. Within 24 hours of collection, mosses were transported to the University of Lethbridge and maintained in growth chambers (I35L, Percival Scientific, Boone, IA; growth chamber space was provided courtesy of E. Schultz and B. McMullen, Department of Biological Sciences, University of Lethbridge). Any shrubs growing in the moss, such as *Betula* and *Ledum*, were trimmed down to approximately 15 cm height above the moss surface for accommodation in the growth chambers. A combination of GROLUX and 'cool white' fluorescent lighting provided 100 $\mu$mol m$^{-2}$ s$^{-1}$ photosynthetic photon flux density (PPFD) at the moss surface for 16 hours per day. Temperature was maintained at 22-24$^\circ$C during illumination and 18-20$^\circ$C during dark periods. *Pleurozium* and *Sphagnum* received 0.5 L and 1.0 L respectively of de-ionized water applied via mist sprayer three times per week. Water table depth from the moss surface was maintained at 5 to 7 cm for *Sphagnum* and 15 to 18 cm for *Pleurozium* to simulate the relative positions of the moss surface above the water table in the field. Mosses collected in August 2004 were used for laboratory experiments conducted during October 2004.

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## 2.3 Instrumentation for spectral reflectance measurements

Spectral reflectance was acquired using two spectroradiometers - the UNI003 'Unispec' Spectral Analysis System (PP Systems, Haverhill, MA) and the Analytical Spectral Devices (ASD) FieldSpec Full Range (FR) instrument, (ASD Inc., Boulder, CO). A comparison of measurements made by these two spectroradiometers is provided in Appendix A. Although differences were found to exist between the measurements from the two instruments (Appendix A), they provided the consistency required for the applications in this thesis. Differences in reflectance were found to be less than one percent with reflectance offsets between the two instruments being relatively constant across the spectrum. Spectral resolution of the Unispec was approximately 10 nm across the usable range between 400 and 1,000 nm. Spectral resolution of the ASD-FR FieldSpec was approximately 3 nm between 350 and 1,000 nm and 10 to 12 nm between 1,000 and 2,500 nm. These resolutions are expressed as full width half maximum (FWHM). This expression of spectral resolution indicates the ability of the instrument to respond to narrow band monochrome radiation. It indicates how large the bandwidth of the radiometer's response is (at half the value detected to be the bandwidth of maximum response) compared to the actual bandwidth of incident radiation (Hatchell 1999). Data from both spectroradiometers were interpolated to 1 nm bandwidths for calculation of reflectance to be consistent with methods used in related studies (Gamon et al. 1993, Lovelock and Robinson 2002, Stylinski et al. 2002, Houston 2004) even though the spectral resolution of the instruments is less precise than 1 nm. Data collected by the ASD-FR FieldSpec were automatically output from the instrument at 1 nm bandwidths.

Post-processing software (MultiSpec 5.0, Faiz Rahman, Ball State University) was used for Unispec data interpolation to 1 nm bandwidths. Laboratory measurements utilized a calibration panel (Spectralon, Labsphere Inc, North Sutton, NH), a diffuse reflective surface, made of ~99% reflective polytetrafluoroethylene (PTFE). Field measurements utilized a 3.2 cm radius PTFE calibration disc (UNI420, PP Systems, Amesbury, MA). Prior to acquiring radiance measurements, spectroradiometer sensitivity (optimization or integration time) was automatically adjusted to the maximum radiation intensity conditions with the foreoptic aimed at the PTFE calibration surface. Each target radiance measurement was preceded with dark current noise measurement (and subtraction) and a measurement of the calibration panel or disc. Measurements were automatically recorded as the average of 10 scans. Reflectance was calculated as the measured radiation intensity reflected by the moss target (radiance), divided by the radiation intensity incident on the target from the illumination source (irradiance) yielding a unitless value (equation 1).

$$\text{Reflectance} = \frac{\text{Target Radiance}}{\text{Irradiance}} \tag{1}$$

For each target radiance measurement, irradiance was calculated by dividing a measurement of the diffuse reflected energy from the calibration panel (PTFE radiance) by known PTFE reflectance (equation 2) (Peddle et al. 2001a).

$$\text{Irradiance} = \frac{\text{PTFE radiance}}{\text{PTFE Reflectance calibration factor}} \tag{2}$$

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In the field, reflectance measurements were acquired at mid-day (1100 – 1400 h) during cloud free conditions. These criteria existed whether using natural and artificial irradiance.

## 2.4 Laboratory dry down experiments

Laboratory experiments were conducted to investigate the response of net $CO_2$ exchange to changes in water content using spectral vegetation indices to provide insights to moss biochemical capacity, photochemical efficiency, and water content. Net $CO_2$ exchange, reflectance, and water content of *Pleurozium* and *Sphagnum* were measured during dry-down experiments similar to those conducted by Murray et al. (1989), Williams and Flanagan (1996), and Schipperges and Rydin (1998). Five replicate moss samples were removed from the moss collections in the growth chambers. All replicates for each species were clipped from the moss surfaces within a 10 cm radius of each other. *Sphagnum* samples contained 90-95% *Sphagnum teres* (Schimp.) Aongstr. *ex* C. Hartm. and 5-10% *Sphagnum angustifolium* (C. Jens *ex* Russ.) C. Jens in Tolf based on the number of capitula. *Sphagnum teres* forms small cushions or lawns below the larger *Sphagnum fuscum* and *Sphagnum angustifolium* hummocks. All *Pleurozium* samples were purely *Pleurozium schreberi*. (Brid.) Mitt. (J. Doubt, Prairie and Northern Plant Diversity Centre, Devonian Botanic Garden, University of Alberta confirmed identification of species). Mosses appeared green and healthy at the time of clipping and replicate samples were of consistent colour and stem density. Each clipping was acquired by gently countersinking an inverted glass cuvette (5 cm diameter) into the moss lawn (Figure 3). Dissection scissors were used to trim vertically into the moss surface around the outside edge of the cuvette and a 1 cm deep portion of the upper green layer was

excised away from the peat layer. Litter debris was removed from the clipping. The moss

clipping was placed upright in its natural orientation in a pre-weighed 5 cm diameter, 1

cm deep glass cuvette yielding an optically thick layer of photosynthetic tissue. The

cuvette was then filled with de-ionized water, submerging the moss. After 10 minutes,

excess water was poured from the cuvette and the moss sample was put in the gas

exchange chamber.

The water-jacketed gas exchange chamber, designed to accommodate a 5 cm

diameter cuvette (see details in Williams and Flanagan 1996), enabled measurement of

$CO_2$ exchange and water vapour flux in moss samples (Figure 3). Compressed air with

known $CO_2$ concentration was supplied at constant rate to the chamber using a mass flow

controller (type: 246C, MKS instruments, Wilmington, MA). Carbon dioxide and water

vapor concentration of the air exiting the chamber was measured using an infrared gas

analyzer (LI 6262, Licor, Lincoln, NE). Moss temperature was monitored using four

copper-constantan (type T) thermocouples placed in contact within the moss. Moss

temperature was maintained at 25°C using a temperature controlled water bath (1160A,

Polyscience or VWR Scientific, Niles, IL) that circulated water through the hollow walls

of the glass chamber. Illumination was provided by a 300 W halogen lamp that was

filtered through a 'hot mirror' to reduce heat load on the chamber. Photosynthetic photon

flux density (PPFD) inside the chamber was monitored with a photodiode (Hamamatsu,

Bridgewater, N.J.) calibrated using a quantum sensor (LI-189, Licor, Lincoln, NE). One-

minute averages of PPFD, moss temperature, $CO_2$, and $H_2O$ vapour concentrations in the

air stream exiting the chamber were recorded using a data logger (CR10, Campbell

Scientific, Edmonton, Alberta) interfaced with computer software (PC208w, Campbell

Scientific, Edmonton, Alberta). Moss temperature, PPFD, and $CO_2$ concentration of the air stream entering the chamber were maintained constant during dry down experiments as summarized in Table 2. Photosynthetic photon flux density was maintained at the light saturation point for photosynthesis as determined from prior measurements of physiological light response curves. The $CO_2$ concentration of the source compressed air supply tank was approximately equal to ambient field conditions and was constant at the beginning and the end of all experiments. This was checked by measuring the $CO_2$ concentration in the system while the moss sample was removed. Moss temperature was maintained throughout the experiments at 25°C because previous studies indicated that this was the optimal temperature for moss photosynthesis (Harley et al. 1989, Williams and Flanagan 1998). Net photosynthetic rate was calculated from data acquired with this open differential gas exchange system as the rate of depletion of $CO_2$ per square meter of moss surface (based on the 5 cm diameter cuvette) per second using equation 3 (Field et al. 1989).

Table 2: Experimental conditions for mosses in the gas exchange chamber during the laboratory dry-down experiments.

|  | *Pleurozium* | *Sphagnum* |
|---|---|---|
| PPFD, $\mu$mol m$^{-2}$ s$^{-1}$ | 400 | 500 |
| Air flow rate, L min$^{-1}$ | 0.40 | 0.40 |
| Chamber inflow [$CO_2$], ppm | 379.0 | 397.1 |
| Moss temperature, $^{\circ}$C | 25 | 25 |

$$A = \frac{Ue * Mfe - Uo * Mfo}{S} \qquad (3)$$

*Where:*

A = Net $CO_2$ exchange ($\mu$mol $CO_2$ m$^{-2}$ s$^{-1}$), positive = net uptake

Ue = $CO_2$ concentration of air entering chamber ($\mu$mol $CO_2$ mol$^{-1}$ air)

Uo = $CO_2$ concentration of air exiting chamber ($\mu$mol $CO_2$ mol$^{-1}$ air)

S = moss area (cuvette size = 0.00196 m$^2$)

Mfe = Molar flow rate of air entering chamber (mol s$^{-1}$)

$$Mfe = \frac{P * f}{R * T}$$

*Where*: P = atmospheric pressure (bar)

f = air flow rate (L s$^{-1}$)

R = gas constant (0.08314 L bar mol$^{-1}$ K$^{-1}$)

T = absolute temperature (K) of air

Mfo = Molar flow rate of air exiting chamber (mol s$^{-1}$)

$$Mfo = Mfe * \frac{1 - He * 10^{-3}}{1 - Ho * 10^{-3}}$$

*Where*: He = water vapour concentration entering chamber (mmol mol$^{-1}$)

Ho = water vapour concentration exiting chamber (mmol mol$^{-1}$)

During laboratory dry down experiments, reflectance measurements were acquired with the illumination source and spectroradiometer foreoptic fixed in position above a removable stage (Figure 3). The moss sample stage was removable so it could be switched with the calibration panel. For laboratory dry down experiments, 500 $\mu$mol m$^{-2}$ s$^{-1}$ PPFD was provided to the moss surface from a 45° zenith angle using a 500 W halogen lamp 30 cm from the moss target. The Unispec fibre optic cable (UNI684 2 m single fibre with 100 mm STR ferrule, PP systems, Haverhill, MA) had a view angle of 28° and was attached at nadir 6 cm above the target yielding a field of view (FOV) diameter of 3 cm. The ASD-FR was equipped with an 18° lens mounted next to the Unispec foreoptic also 6 cm above the target resulting in an FOV diameter of 2 cm.

Prior to moss reflectance measurements, the PTFE calibration panel reflectance was measured. Following calibration measurements, the PTFE calibration panel was replaced with a non-reflective black panel with a slot for a 5 cm diameter cuvette. Once the moss net photosynthesis rate reached steady state for the given conditions (>12-15 minutes), the moss was removed from the chamber and immediately measured by the two spectroradiometers. To ensure consistent alignment of the moss for each reflectance measurement, a marker on the side of the cuvette was aligned with a marker on the black panel. The time lag between recording photosynthesis and reflectance data with both spectroradiometers was less than 30 seconds. Next, the moss (plus cuvette) was weighed using a top loading balance (PJ400, Mettler Instruments or Mettler Toledo, Zurich) and returned to the gas exchange chamber. A series of measurements was conducted on the moss sample about once per hour as it dried down. The end of the experiment occurred when net $CO_2$ exchange rate declined below zero. The moss was then placed in a drying

oven at 60°C for 48 hours. Moss dry weight was recorded enabling comparison of photosynthesis and reflectance data to water content expressed as the ratio of moss fresh weight to moss dry weight.

## 2.5 Field studies of moss water content and reflectance

Measurements were acquired to test if the relationship between WBI and water content measured under laboratory conditions could be observed in the field. Water content and reflectance data for undisturbed *Sphagnum teres* and *Pleurozium schreberi* were collected using two methods at the Western Peatland flux station with the Unispec spectroradiometer during the growing season of 2004. One of these methods used solar illumination and the other used artificial illumination.

When using direct solar illumination, reflectance measurements were made when PPFD was saturating for photosynthesis. The spectroradiometer foreoptic was suspended by hand, with nadir orientation, 6 cm above the moss surface. The sensor angular FOV was 28° yielding a surface FOV diameter of 3 cm. On October 14, 2004, twenty measurements were conducted per moss species, 10 of these moss samples were at their natural water content and 10 were artificially moistened using a mist spray bottle the evening prior to the day of measurement. This was done to investigate the effect of short term changes in water content on reflectance of undisturbed mosses. Measurements of moss water content were conducted after acquiring reflectance data. For water content measurements, moss samples were collected using the same procedure as in the laboratory experiments. The moss samples were transported in sealed plastic bags and fresh weight was measured using a top loading balance (PJ400, Mettler Instruments,

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Zurich) within 10 minutes of collection. The mosses were then dehydrated at 60°C

overnight using a food dehydrator. Samples were then re-weighed for determination of

water content.

In addition to the method described above, an alternative methodology using

artificial illumination was tested to improve control of illumination conditions. This

reflectance data collection involved a non-conventional approach to control for variable

field illumination conditions including sun angle, sensor to surface orientation, and

attenuation of light by overstorey vegetation. Field and laboratory measurements were

acquired using a 7 W internal halogen light source and bifurcated cable (Mini Foreoptic,

PP systems, Haverhill, MA) integrated with the Unispec to provide controlled

illumination measurement of small targets. This method of measuring moss surfaces was

an adaptation of a method for measuring reflectance of individual leaves described by

Gamon and Surfus (1999). Appendix B provides details of these tests including

limitations encountered and suggested improvements.

## 2.6 Bi-directional reflectance

Due to the weak correlation observed between WBI and water content in the field

(see Results section 3.2.1), it was hypothesized that variation in illumination and sensor

geometry may have been a factor influencing the results. To test this hypothesis, the

BRDF was measured using the University of Lethbridge Goniometer System (ULGS)

(Coburn and Peddle 2005). Using the ULGS, measurements of reflectance were made in

the laboratory from many viewing angles while the light source remained in a fixed

position. The bi-directional reflectance distribution function of *Pleurozium schreberi* was interpolated from these laboratory measurements collected using a goniometer (designed and built by C. Coburn, Department of Geography, University of Lethbridge) described by Coburn and Peddle (2005) and shown in Figure 4.

*Pleurozium* (water content: 10.9 fresh weight / dry weight), contained in a sample bin (60 cm x 30 cm x 20 cm deep), was placed underneath the goniometer and spectral measurements were recorded with the ASD-FR FieldSpec spectroradiometer by revolving the goniometer arch $360^{\circ}$ around the target at $10^{\circ}$ increments. A 500 W halogen lamp, oriented 1.5 m from the moss target at $45^{\circ}$ from nadir, illuminated the moss from a $150^{\circ}$ azimuth. The ASD-FR FieldSpec foreoptic ($5^{\circ}$) was attached to the goniometer arch 49 cm from the target yielding a FOV at nadir of 4.3 cm. The ASD-FR FieldSpec recorded target radiance at $10^{\circ}$ zenith increments from $-60^{\circ}$ to $60^{\circ}$ zenith by adjusting the position of the foreoptic bracket along the arch. This was repeated at each $10^{\circ}$ azimuth increment between azimuths of $0^{\circ}$ and $180^{\circ}$ as the arch was rotated clockwise. The goniometer thus ensured the foreoptic was kept a constant distance from the target (49 cm) and was always aimed directly at the same point on the moss surface. Moss radiance measurements were calibrated to PTFE calibration panel radiance measurements recorded from nadir prior to each set of 13 azimuthal measurements. This procedure provided 234 measurements of the same target from different viewing positions and with consistent illumination intensity and angle. Following these measurements, an additional set of 13 measurements with identical foreoptic position to the first set were recorded at the end of the goniometer rotation to check for agreement between measurements made at the beginning and end of the measurement cycle (approximately 30 minutes). A post-

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processing macro written in Visual Basic for MS Excel (by G. Dooper, Department of

Geography, University of Lethbridge), calculated reflectance spectra for all 234 data files

and enabled user-specified extraction of wavelength bands. From this, WBI was

calculated for all viewing angles and plotted on an XY scatterplot to aid comparison of

WBI values measured from different zenith angles and azimuths.

Figure 4: Photograph of the goniometer system for measuring bi-directional reflectance properties showing the halogen light source (1), ASD-FR spectroradiometer (2) with foreoptic (3) attached to the goniometer arch (4), and two interchangeable targets: *Pleurozium* moss (5) and PTFE calibration panel (6).

# 3 RESULTS

## 3.1 Laboratory dry down experimentation

3.1.1 Net photosynthesis measurements

As expected, net photosynthesis rates varied in a non-linear fashion relative to changes in water content (Figure 5). These physiological response curves showed optimal water contents for maximum net $CO_2$ uptake for *Sphagnum* between 9 and 12 (a unitless expression of fresh weight divided by dry weight) and between 5 and 7 for *Pleurozium*. All replicates showed very similar patterns. Water contents higher than these optimal values resulted in inhibition of net photosynthesis and water contents lower than optimum resulted in dramatic declines in net photosynthesis. Water holding capacity of *Sphagnum* (30 fresh weight / dry weight) was higher than that of *Pleurozium* (~14), however the time required for both species to dry down completely from their highest water content was approximately fourteen hours.
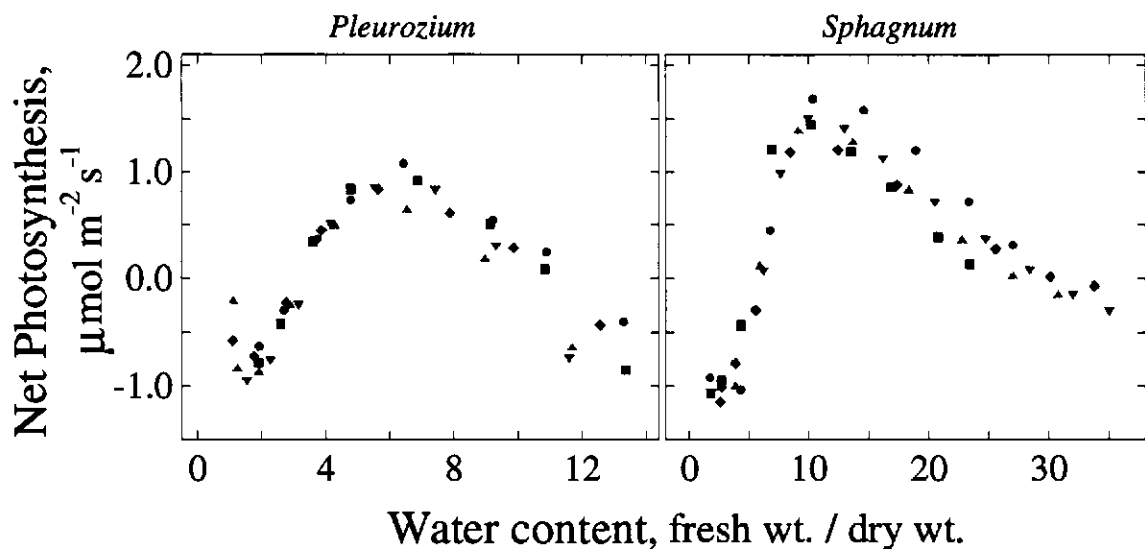
Figure 5: Influence of changes in water content on net photosynthesis rate in *Sphagnum* and *Pleurozium*. Symbols represent separate replicated dry down experiments: replicate 1 ●, 2 ■, 3 ◆, 4 ▲, and 5 ▼.

3.1.2 Reflectance measurements

Moss reflectance measurements and vegetation index calculations were consistent between the two spectroradiometers (Appendix A). All reflectance results presented in this chapter were calculated from Unispec spectroradiometer measurements except for the spectral response curves shown in Figures 6 to 9. Data from the ASD-FR FieldSpec were presented to illustrate the effects of dry down on spectral reflectance between 500 and 2,500 nm (Figures 6 to 9). Unispec data was emphasized because it was the spectroradiometer used for both laboratory and field data collection due to its portability and potential in future work to provide controlled illumination in the field with its internal halogen light source. During the dry down experiments, reflectance increased across most of the measured spectral range including the visible, near infrared, and

middle infrared portions of the spectrum (Figures 6 to 9). From the beginning to the end

of moss dry down, the greater rate of increase in reflectance at 970 nm relative to 900 nm

was readily observable (Figures 6 and 8). *Sphagnum* exhibited greater reflectance

increases across the entire spectrum than *Pleurozium* and both species exhibited slight

shifts in peak visible reflectance to longer wavelengths (Figures 7 and 9).
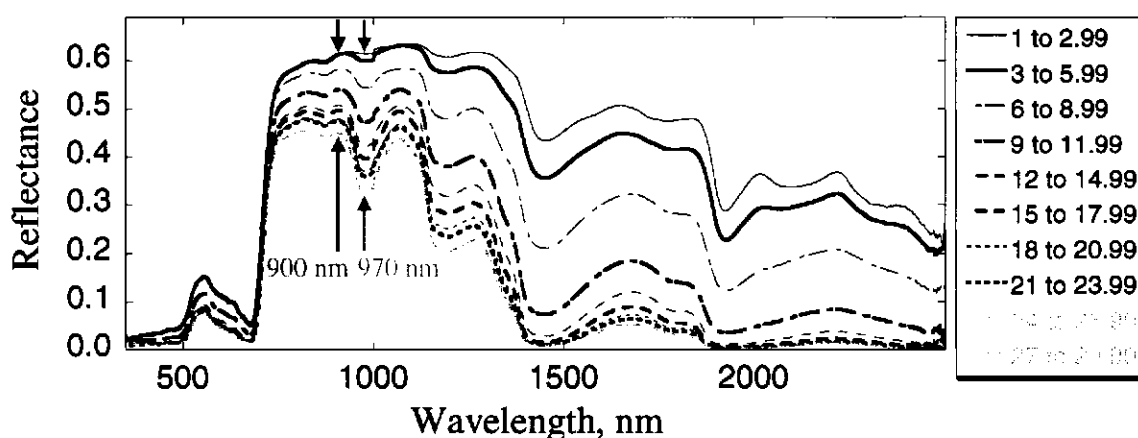


Figure 6: Influence of changes in water content on the spectral distribution of reflectance of *Sphagnum* moss in the laboratory. Lines (quantified in the legend) represent mean reflectance (ASD-FR FieldSpec) for specified water content (fresh weight / dry weight) ranges averaged from 5 dry down experiments. Vertical arrows highlight the change in reflectance at 900 nm (left arrow) relative to the change in reflectance at 970 nm (right arrow).
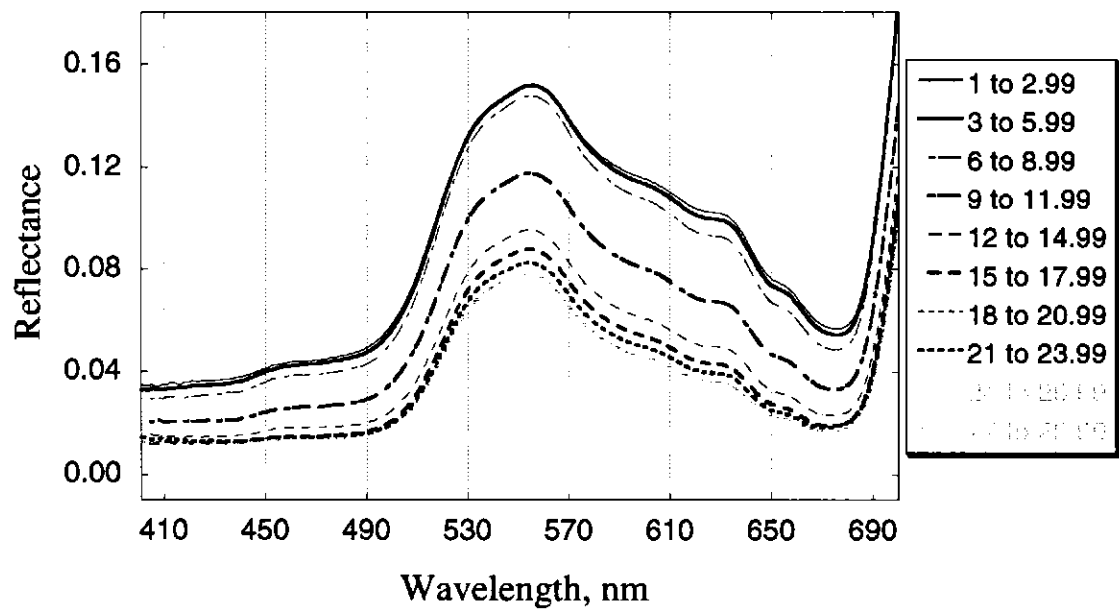
Figure 7: Influence of changes in water content on the spectral distribution of reflectance across the photosynthetically active radiation portion of the spectrum of *Sphagnum* moss in the laboratory. Lines (quantified in the legend) represent mean reflectance (ASD-FR FieldSpec) for specified water content (fresh weight / dry weight) ranges averaged from 5 dry down experiments.
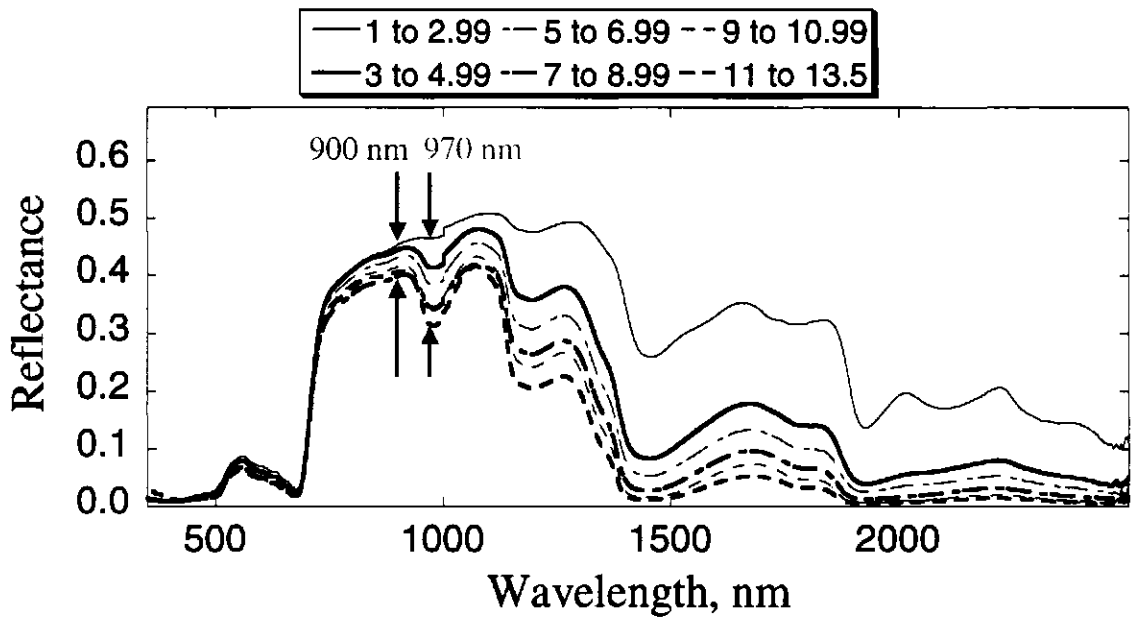
Figure 8: Influence of changes in water content on the spectral distribution of reflectance of *Pleurozium* moss in the laboratory. Lines (quantified in the legend) represent mean reflectance (ASD-FR FieldSpec) for specified water content (fresh weight/dry weight) ranges averaged from 5 dry down experiments. Vertical arrows highlight the change in reflectance at 900 nm (left arrow) relative to the change in reflectance at 970 nm (right arrow).
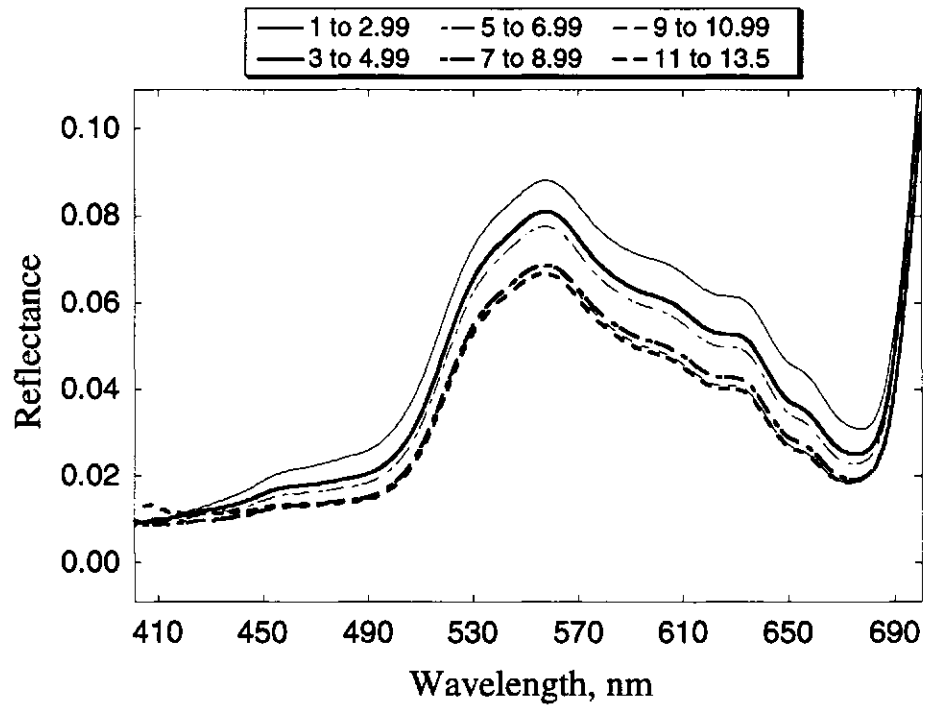
Figure 9: Influence of changes in water content on the spectral distribution of reflectance across the photosynthetically active radiation portion of the spectrum of *Pleurozium* moss in the laboratory. Lines (quantified in the legend) represent mean reflectance (ASD-FR FieldSpec) for specified water content (fresh weight/dry weight.) ranges averaged from 5 dry down experiments.

The response of NDVI to changes in water content (Figure 10) agreed with

hypothesized patterns. At water contents above the optimum for net photosynthesis,

NDVI was high and equal to values at optimum water content. As water content dropped

below levels optimal for net photosynthesis, NDVI declined linearly. Two differences in

this NDVI response were observed between the two moss species. First, NDVI values

were less variable between replicates for *Sphagnum* relative to *Pleurozium*. Second,

*Sphagnum* replicates had slightly higher maximum and slightly lower minimum NDVI

values as compared to *Pleurozium*, although NDVI values were strongly overlapping

between the two species. Due to the combination of these two differences, the mean

change in NDVI between the beginning and end of the experiment in *Sphagnum* was

greater (~2 times) than that observed for *Pleurozium* (Table 3). For *Sphagnum*,

chlorophyll index responded to water content in a manner similar to that of NDVI,

although higher variability existed between replicates. For *Pleurozium schreberi*, a moss

more resistant to water loss (Williams and Flanagan 1998), CI remained constant during

dry down except for a slight increase at water contents below 3 (fresh weight / dry

weight).

Figure 10: Influence of changes in moss water content on NDVI and CI in the laboratory. Symbols represent separate replicated dry down experiments: replicate 1 ●, 2 ■, 3 ◆, 4 ▲, and 5 ▼.

Table 3: Average NDVI values for *Sphagnum* and *Pleurozium* replicates at the start and end of the dry down experiment (n=5).

|  |  | Mean | Standard deviation |
|---|---|---|---|
| *Sphagnum* | Start NDVI | 0.922 | 0.008 |
|  | End NDVI | 0.817 | 0.012 |
|  | Start - end | 0.105 | 0.008 |
| *Pleurozium* | Start NDVI | 0.903 | 0.018 |
|  | End NDVI | 0.853 | 0.026 |
|  | Start - end | 0.050 | 0.011 |

In contrast to hypothesized patterns, PRI appeared to remain constant despite changes in net photosynthesis and light use efficiency resulting from reductions in moss water content (Figure 11). For *Sphagnum*, PRI was consistently higher and exhibited less variance between replicates relative to PRI values of *Pleurozium*. Simple linear regression was calculated for PRI and water content data with water contents higher than optimum for net photosynthesis. This selection process eliminated data related to the down regulation of photosynthesis due to desiccation. This was done to investigate if reductions in LUE due to $CO_2$ limitation, caused by an enhanced diffusion barrier, would result in the hypothesized reduction in PRI. For *Pleurozium*, the highest water content value observed prior to peak photosynthesis was 6 (fresh weight / dry weight). For *Sphagnum* this water content value was 12. Even after eliminating the PRI and water

content bivariate pairs below this value for *Pleurozium*, none of the regression line slopes for PRI versus water content were statistically significantly different from zero ($p>0.05$). This was the result when replicates were assessed on an individual and a combined basis (Table 4). Two of the five *Sphagnum* replicates and the combination of all replicates had regression line slopes statistically significantly different from zero ($p<0.05$) (Table 5), although all slope values were positive, opposite the hypothesized trend. For both mosses, the differences in PRI between replicates resulted in a high level of data variability as indicated by the scatter in Figure 11 and by low correlation coefficients for replicates on a combined basis (Tables 4 & 5).



Figure 11: Influence of changes in moss water content on PRI in the laboratory. Symbols represent separate replicated dry down experiments: replicate 1 ●, 2 ■, 3 ◆, 4 ▲, and 5 ▼.

Table 4: Least squares linear regression analysis results comparing PRI versus water content for replicated dry down experiment measurements of *Pleurozium*. For this analysis, bivariate sets with water contents less than 6 (fresh weight / dry weight) were not included. None of the datasets had regression line slopes statistically significantly different from zero.

| Replicate # | Regression slope significance | Linear equation | Coefficient of determination ($r^2$) |
|---|---|---|---|
| 1 | $F = 0.04$, df = 5, $p > 0.05$ | $y = -0.0002x - 0.07$ | 0.008 |
| 2 | $F = 0.24$, df = 5, $p > 0.05$ | $y = 0.0006x - 0.06$ | 0.050 |
| 3 | $F = 0.34$, df = 4, $p > 0.05$ | $y = -0.0008x - 0.08$ | 0.080 |
| 4 | $F = 0.46$, df = 4, $p > 0.05$ | $y = -0.0007x - 0.08$ | 0.100 |
| 5 | $F = 0.33$, df = 5, $p > 0.05$ | $y = -0.0003x - 0.06$ | 0.060 |
| All 5 combined | $F = 0.12$, df = 31, $p > 0.05$ | $y = -0.0004x - 0.07$ | 0.004 |

Table 5: Least squares linear regression analysis results comparing PRI versus water content for replicated dry down experiment measurements of *Sphagnum*. For this analysis, bivariate sets with water contents less than 12 (fresh weight / dry weight) were not included. Datasets marked with an asterisk had regression line slopes statistically significantly different from zero.

| Replicate # | Regression slope significance | Linear equation | Coefficient of determination $(r^2)$ |
|---|---|---|---|
| 1* | $F = 16.82, df = 5, p < 0.01$ | $y = 0.0009x - 0.035$ | 0.771 |
| 2 | $F = 1.77, df = 5, p > 0.05$ | $y = 0.0006x - 0.05$ | 0.262 |
| 3 | $F = 3.19, df = 9, p > 0.05$ | $y = -0.0004x - 0.04$ | 0.260 |
| 4 | $F = 3.36, df = 7, p > 0.05$ | $y = 0.0007x - 0.04$ | 0.320 |
| 5* | $F = 20.62, df = 12, p < 0.01$ | $y = 0.0006x - 0.04$ | 0.632 |
| all 5 combined* | $F = 11.49, df = 46, p < 0.01$ | $y = 0.0006x - 0.04$ | 0.200 |

A strong positive relationship was observed between WBI and moss water content (Figure 12) as hypothesized. For *Sphagnum*, the slope of the relationship between WBI and water content began to change (perhaps because of saturation of WBI) at water contents above 20, weakening an otherwise strong linear relationship. Fewer than 2% of the water content values measured during the 2004 growing season at the Western Peatland were greater than 22. This supported the exclusion of bivariate data pairs with water content values greater than 22 for regression analysis. All regression slopes between WBI and water content for all *Pleurozium* and *Sphagnum* replicates were significantly different from zero ($p < 0.05$) and coefficients of determination were greater than 0.97 (Tables 6 and 7). The slope of the WBI versus water content relationship for combined replicates was higher for *Pleurozium* and the y-axis intercept was lower compared to *Sphagnum*.

Since WBI and net photosynthesis rate were strongly related to water content, WBI was also a good predictor of moss net photosynthesis rate under controlled laboratory conditions (Figure 13). Based on the coefficients of determination for fourth order polynomial regressions fit to the data, greater than 85% of the variability in net photosynthesis rate could be accounted for by the variability in WBI using these regressions.
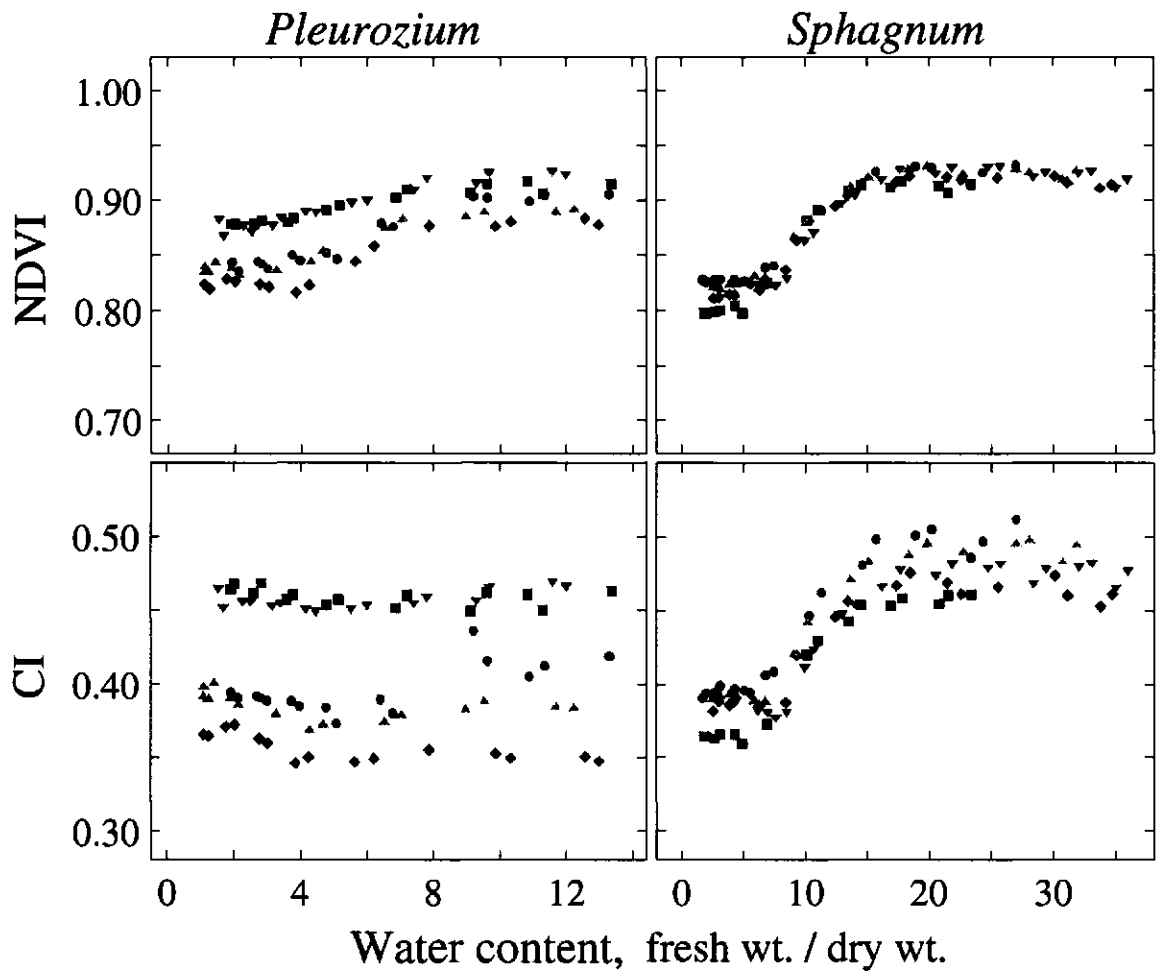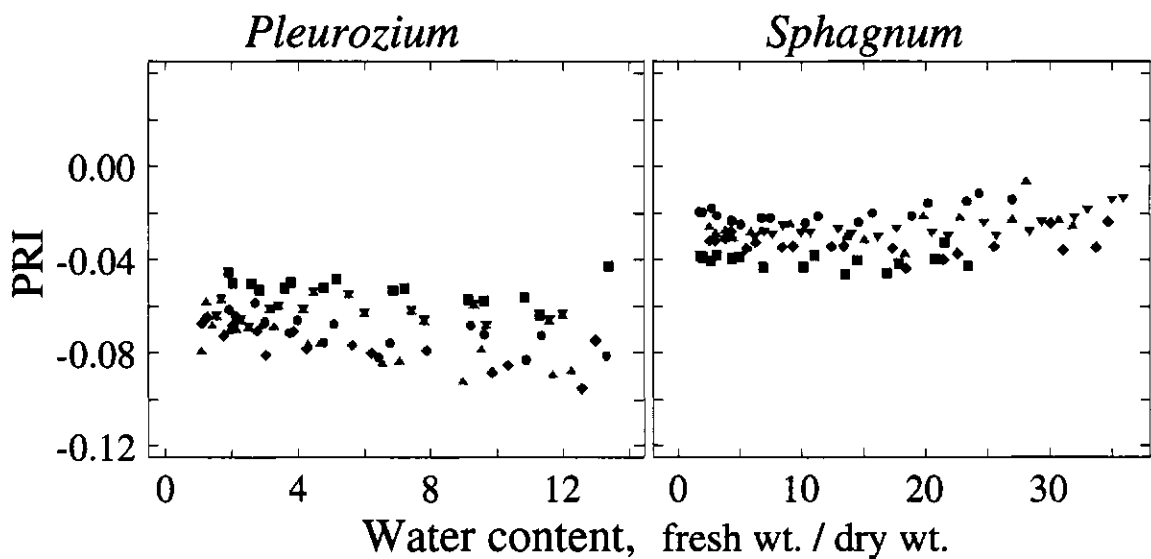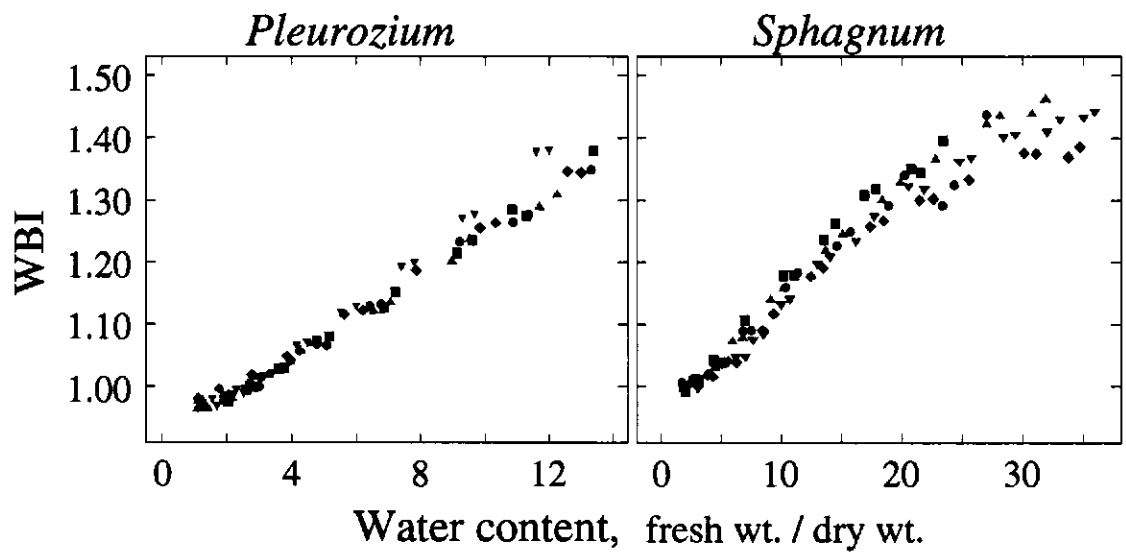
Figure 12: Influence of changes in moss water content on WBI in the laboratory.
Symbols represent separate replicated dry down experiments: replicate 1 ●, 2 ■, 3 ◆, 4 ▲, and 5 ▼.

Table 6: Least squares linear regression analysis results comparing WBI versus water content for replicated dry down experiment measurements of *Pleurozium*. Datasets marked with an asterisk had regression line slopes statistically significantly different from zero.

| Replicate # | Regression slope significance | Linear equation | Coefficient of determination ($r^2$) |
|---|---|---|---|
| 1* | $F = 2816.3$, $df = 13$, $p < 0.01$ | $y = 0.0329x + 0.913$ | 0.998 |
| 2* | $F = 3383.3$, $df = 13$, $p < 0.01$ | $y = 0.0344x + 0.905$ | 0.996 |
| 3* | $F = 3192.5$, $df = 13$, $p < 0.01$ | $y = 0.0325x + 0.929$ | 0.996 |
| 4* | $F = 2762.2$, $df = 14$, $p < 0.01$ | $y = 0.0311x - 0.927$ | 0.995 |
| 5* | $F = 3317.8$, $df = 14$, $p < 0.01$ | $y = 0.0401x - 0.895$ | 0.996 |
| All 5 combined* | $F = 4275.2$, $df = 75$, $p < 0.01$ | $y = 0.0339x + 0.916$ | 0.983 |

Table 7: Least squares linear regression analysis results comparing WBI versus water content for replicated dry down experiment measurements of *Sphagnum*. For this analysis, bivariate sets with water contents greater than 22 (fresh weight / dry weight) were not included. Datasets marked with an asterisk had regression line slopes statistically significantly different from zero.

| Replicate # | Regression slope significance | Linear equation | Coefficient of determination ($r^2$) |
|---|---|---|---|
| 1* | $F = 1606.3, df = 12, p < 0.01$ | $y = 0.0182x + 0.963$ | 0.993 |
| 2* | $F = 903.9, df = 13, p < 0.01$ | $y = 0.0195x + 0.962$ | 0.986 |
| 3* | $F = 1086.1, df = 11, p < 0.01$ | $y = 0.0171x + 0.951$ | 0.990 |
| 4* | $F = 2434.5, df = 10, p < 0.01$ | $y = 0.0192x + 0.956$ | 0.996 |
| 5* | $F = 571.6, df = 10, p < 0.01$ | $y = 0.0187x + 0.933$ | 0.983 |
| all 5 combined* | $F = 2190.4, df = 64, p < 0.01$ | $y = 0.0183x + 0.956$ | 0.972 |
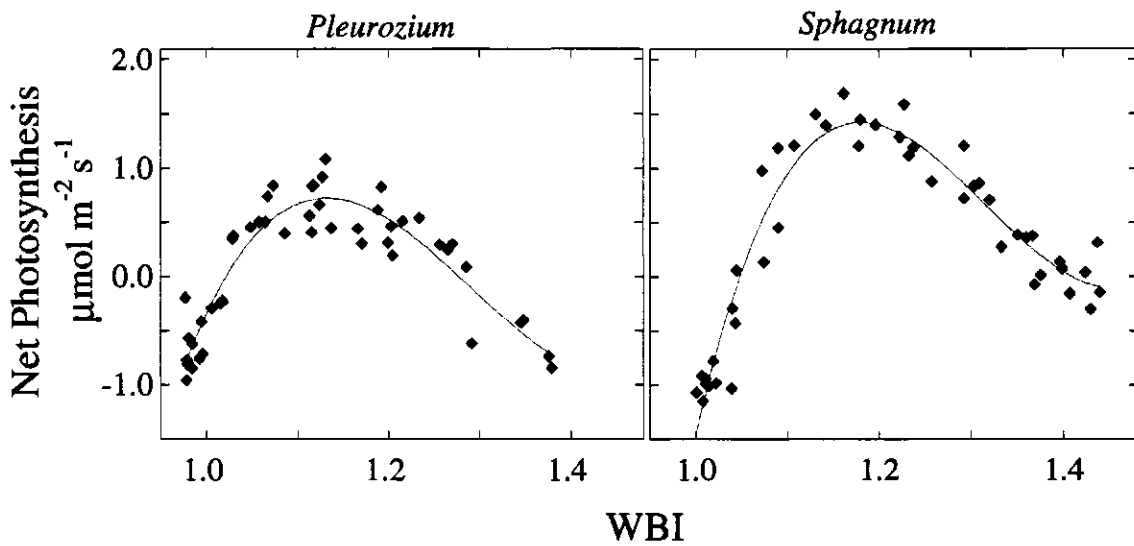
Figure 13: Comparison of net photosynthetic rate to WBI in the laboratory for *Pleurozium* (y = $100.8x^3 - 390.7x^2 - 497.1x - 207.5$, $r^2$=0.855) and *Sphagnum* (y = $150.0x^3 - 592.5x^2 + 771.8x - 330.8$, $r^2$=0.916) under constant PPFD, temperature, and $CO_2$ concentration. All five experiments combined.

## 3.2 Field studies of moss water content and reflectance

### 3.2.1 Reflectance using solar illumination

Field measurements using solar illumination produced a range of WBI values

overlapping with values acquired in the laboratory (Figure 14). *Pleurozium* and

*Sphagnum* water contents in the field were within the range measured in the laboratory

and the field water contents exhibited a smaller range. For both species, simple linear

regression analyses were conducted for WBI relative to natural and artificially enhanced

water contents and the combination of both. All coefficients of determination between

WBI and water content in the field were less than 0.1 and none of the regression line

slopes were statistically significantly different from zero (p>0.05). Using a normalized

form of WBI, with the difference between reflectance at 900 and 970 nm divided by their sum, did not yield improvements in the relationship with water content.

Contrary to expectations, NDVI, CI, and PRI values were different in the field relative to the laboratory. Values for NDVI, CI and PRI were generally lower in the field than in the lab for both *Pleurozium* and *Sphagnum* (Figure 15). Also different from observations in the laboratory, reflectance measurements in the field resulted in lower PRI values for *Sphagnum* than for *Pleurozium*. The photochemical reflectance index exhibited more variability between replicates in the field for both moss species.
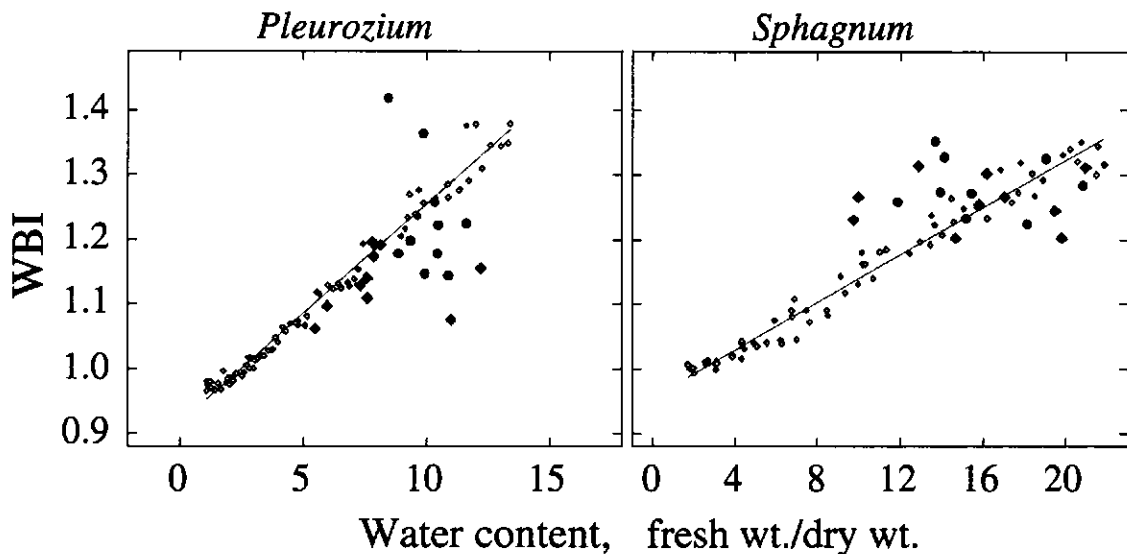


Figure 14: Influence of changes in moss water content on WBI in the field using solar illumination for reflectance measurements. Data points represent field WBI values at natural water content ◆ and at artificially increased water content ●. The line and associated data points ——◆—— represents the line of best fit from laboratory results of WBI versus water content: y = 0.0339x+0.916 for *Pleurozium* and y = 0.0183x + 0.956 for *Sphagnum*.

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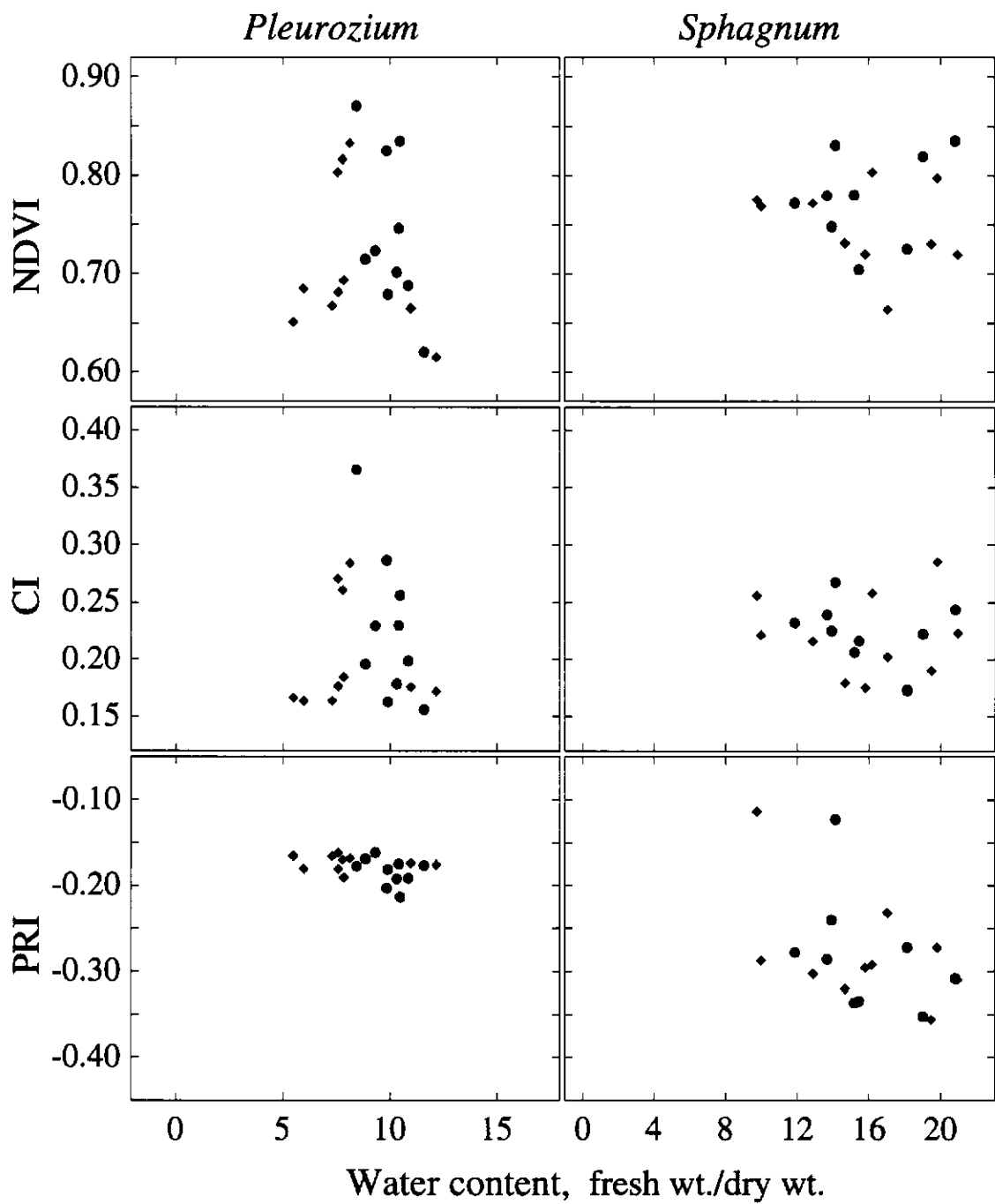
Figure 15: Influence of changes in moss water content on NDVI, CI, and PRI in the field using solar illumination for reflectance measurements. Data points represent index values at natural water content ✦ and at artificially increased water content ●.

3.2.2 Reflectance using artificial illumination

Although this method requires further refinement to acquire reliable measures of absolute target reflectance, it did provide regression slopes for WBI and water content data in *Sphagnum* that were not statistically significantly different from laboratory data. This was not true for *Pleurozium*. These measurements on *Pleurozium* were likely complicated by radiation transmission through the moss canopy and possibly low artificial illumination intensity as described in Appendix B. It was concluded that to improve this approach, two conditions would need to be met. First, further steps need to be taken to prevent transmission of ambient radiation through the moss canopy. Second, the intensity of the artificial illumination would need to be increased substantially.

## 3.3 Bi-directional reflectance

Measurements of bi-directional reflectance using an ASD-FR FieldSpec spectroradiometer mounted on a goniometer showed variability in WBI depending on view angle (Table 8 & Figure 16). In Table 8, the first three columns describe values of reflectance at 900 nm, reflectance at 970 nm, and WBI acquired from all hemispherical measurements except one angle (azimuth: $180°$, zenith: $20°$) due a problematic file. The remaining columns describe WBI values recorded from nadir only (WBI n), all prescribed angles excluding nadir recordings (WBI nn), from 60 degrees zenith only (WBI 60), and from 40 degrees zenith only (WBI 40). On the bottom row, *Pleurozium* water content (fresh weight / dry weight) was estimated using the linear regression equation: water content = (WBI-0.916)/0.0339, derived from dry down experiments.

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These estimated water contents are expressed as mean values with standard deviations in parentheses. Actual mean water content was measured from three clippings of the moss and found to be 10.9 with a standard deviation of 0.7.

In Figure 16, for each measurement number, the foreoptic was located at a different zenith and azimuth perspective. The graphics of Figure 16A represent reflectance 900 nm (left) divided by reflectance at 970 nm (middle) resulting in WBI (right) for one target measured from various viewing angle perspectives indicated by the white dots. Figure 16B shows the same dataset with the WBI values for each sequential measurement made while moving the foreoptic. For example, the measurement numbers 1 to 13 on the X-axis were acquired by passing the foreoptic along the goniometer arch over the moss in a straight line azimuth (from $0°$ to $180°$) pausing to measure at $10°$ *zenith* view angle increments from $60°$ (measurement 1) across nadir (measurement 7) and to $60°$ on the other side of nadir (measurement 13). This was repeated for each $10°$ azimuth increment between $0°$ and $180°$ by rotating the goniometer arch horizontally. Each set of 13 measurements at specified $10°$ azimuth increment is indicated on the graph by a scale bar of black and white alternating dashes with the nadir measurement at the middle of each dash. Water content was estimated for each WBI value (second Y-axis) using the laboratory dry down experiment regression equation: estimated water content = (WBI - 0.916) / 0.0339. Actual water content was 10.9 (fresh weight / dry weight).This provided some insight as to what may have been contributing to the difference between laboratory and field results. When viewed from nadir, the calculated WBI value was approximately 1.4 and when viewed from $160°$ azimuth and $30°$ zenith, WBI was 1.46, the maximum observed value. In general, the further the view angle was from nadir, the lower the

measured WBI value. The lowest WBI value, 1.23, occurred when viewed from an azimuth of 150° and a zenith of 60°. Using the best-fit relationship between WBI and water content from dry down experiments shown in Table 6, the highest and lowest WBI values of 1.23 and 1.46 predicted water contents of 9.3 and 16.0 (fresh weight / dry weight) respectively. The actual water content of *Pleurozium* during goniometer measurements was 10.9 (fresh weight / dry weight), which would translate into a WBI value of approximately 1.3 based on the same regression equation. The mean WBI values from the goniometer were higher than this expected WBI value. Only the mean of the WBI values measured from 60° zenith had a standard deviation range that overlapped with the expected WBI (Table 8).

The WBI of *Pleurozium* produced a three dimensional surface of BRDF that resembled the shape of an inverted bowl (Figure 16). In general, higher zenith view angles yielded lower WBI values. This result was caused by approximately equal changes in reflectance for both wavebands but because the reflectance magnitude differed between the two bands, R970 nm was disproportionately elevated relative to R900 nm with increasing zenith angle. For example, for zero degrees azimuth, from nadir (R900/R970 = 0.55/0.4 = 1.375) to 60 degrees zenith (R900/R970 = 0.65/0.5 = 1.3).

Table 8: Reflectance and WBI values of *Pleurozium* measured from many zenith and azimuth angle combinations using an ASD-FR FieldSpec spectroradiometer attached to a goniometer. Details pertaining to this presentation are contained within the text.

|  | R900nm | R970nm | WBI | WBI n | WBI nn | WBI 60 | WBI 40 |
|---|---|---|---|---|---|---|---|
| Mean | 0.58 | 0.43 | 1.35 | 1.39 | 1.35 | 1.32 | 1.35 |
| Stdev | 0.064 | 0.054 | 0.040 | 0.031 | 0.039 | 0.027 | 0.021 |
| SE | 0.004 | 0.003 | 0.003 | 0.007 | 0.003 | 0.006 | 0.005 |
| n | 246 | 246 | 246 | 19 | 226 | 19 | 19 |
| Min | 0.23 | 0.17 | 1.23 | 1.29 | 1.23 | 1.26 | 1.29 |
| Max | 0.85 | 0.65 | 1.46 | 1.43 | 1.46 | 1.36 | 1.40 |
| Range | 0.63 | 0.48 | 0.23 | 0.14 | 0.23 | 0.10 | 0.11 |
| Estimated |  |  | 12.9(1.2) | 13.9(0.9) | 12.8(1.2) | 11.8(0.8) | 12.8(0.6) |
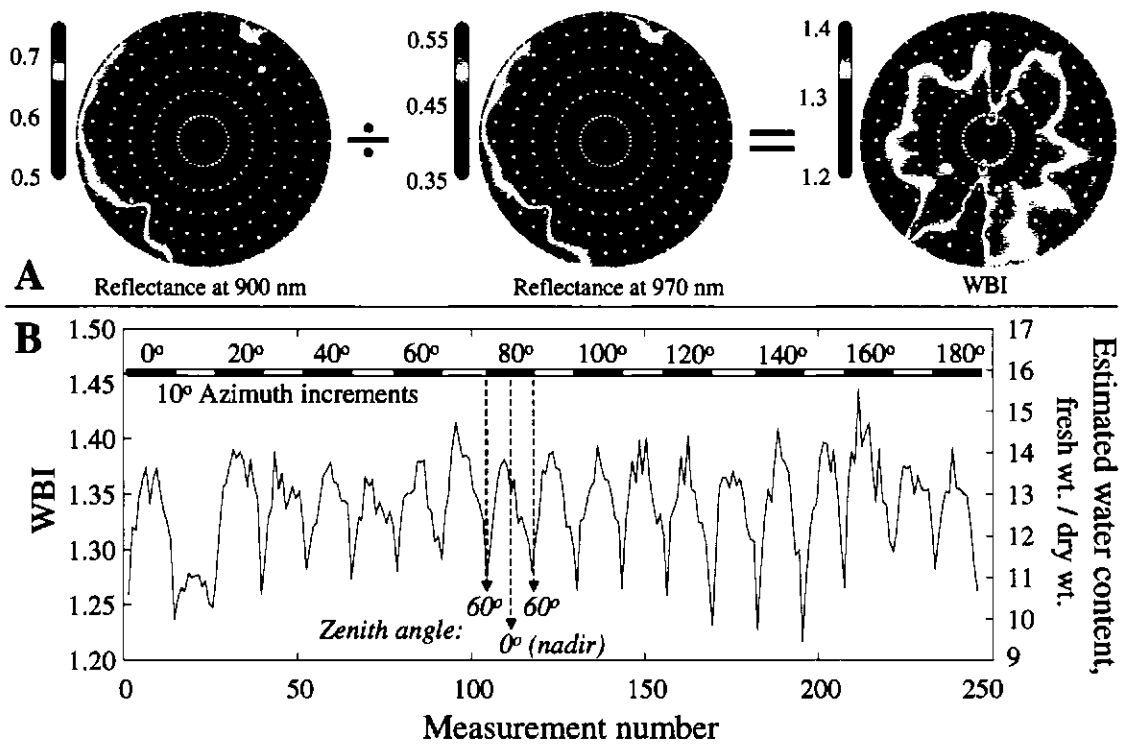
Figure 16: Variability of reflectance and WBI depending on azimuth and zenith view angles for a single *Pleurozium* moss target measured with an ASD-FR FieldSpec spectroradiometer mounted to a goniometer. Details pertaining to this presentation are contained in the text.

# 4 DISCUSSION

This chapter is divided into three sections. The first deals with results that agreed

with predictions, including the physiological, optical reflectance, WBI, NDVI, and CI

responses of moss to changes in water content under controlled laboratory conditions. It

discusses the validity of results by comparing them to established literature. The second

section describes aspects of this research that disagreed with the proposed hypotheses.

The differences between field results and laboratory results for spectral vegetation indices

in relation to moss water content are discussed. This section also describes why bi-

directional reflectance results may account for some of these differences. Finally, the

discrepancy between hypothesized and actual response of PRI to changes in moss water

content is discussed. The final section proposes future research.

## 4.1 Observations in agreement with predictions

### 4.1.1 Net photosynthesis

The response of net $CO_2$ exchange of mosses to changes in water content was

similar to that of previous studies of *Pleurozium* and *Sphagnum,* demonstrating that

excess water inhibited diffusion of $CO_2$ into the cells and sub-optimal water content

resulted in reduction of biochemical capacity for photosynthesis (Murray et al. 1989,

Silvola 1990, Williams and Flanagan 1996, Schipperges and Rydin 1998). Optimal water

contents and maximum net photosynthesis rates (~1.0 $\mu$mol m$^{-2}$ s$^{-1}$ for *Pleurozium* and

~1.5 $\mu$mol m$^{-2}$ s$^{-1}$ for *Sphagnum*) were comparable to values reported in other studies. For

lab experiments with mosses collected from a black spruce forest in Ontario, Williams

and Flanagan (1996) observed optimal moss water contents for maximum net $CO_2$ uptake

of about 6 (fresh weight / dry weight) for *Pleurozium* and about 7 for *Sphagnum* with

associated rates of net $CO_2$ uptake of about 2 $\mu mol\ m^{-2}\ s^{-1}$ and 6 $\mu mol\ m^{-2}\ s^{-1}$ respectively.

For *Sphagnum fuscum* collected in eastern Finland, Silvola (1990) reported net

photosynthesis rates between about 0.5 and 2 $\mu mol\ m^{-2}\ s^{-1}$ for optimal water contents

between 7 and 9 (fresh weight / dry weight). In the field, Murray et al. (1989) reported

optimal water contents between 7 and 11 (fresh weight / dry weight) for *Sphagnum*

*angustifolium* of tussock tundra in Alaska. Murray et al. (1989) found the curved

relationship between net photosynthesis and water content to remain intact but vary

greatly in magnitude with recent precipitation conditions. Following a significant rain

event, they reported net photosynthesis rates to be about three times higher than rates

following a period with relatively little rain (Murray et al. 1989).

4.1.2 Spectral response and water band index in the laboratory

The increase in reflectance between 400 and 2,500 nm with decreased water

content agreed with similar comparisons using *Pleurozium* (Fernandes et al. 1996),

*Sphagnum* (Vogelmann and Moss 1993, Bubier et al. 1997), and vascular plants

(Gausman 1985, Carter 1991). The increase in reflectance at approximately 970 nm

during dry down of vascular plants has been attributed to declining amounts of water

available to directly absorb incident radiation (Carter 1991, Penuelas et al. 1993,

Penuelas and Inoue 1999). It has also been suggested that this phenomenon is not simply

due to absorption of radiation by water (Gausman 1985, Carter 1991, Penuelas et al.

1993). Gausman (1985) and Carter (1991) explained that a rise in near infrared

reflectance related to declining water content was also influenced by cellular and

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intercellular structure changes due to declining leaf turgor. As leaf water content

declined, the amount of surface area between wet cell walls and intercellular air increased

(Carter 1991). These changes caused an increase in the refraction index of the leaf (Carter

1991). When leaf water content was low, incident radiation was more strongly reflected

by these cell wall boundaries (Gausman 1985). This effect may differ between plants

with different cell structures and subsequently affect absorption at 970 nm. For example,

while linear relationships between WBI and water content have been observed in studies

of several species, large differences in best-fit line equations occurred between different

plant types (Sims and Gamon 2003). In this study, linear regression equations were

different between *Pleurozium* and *Sphagnum* suggesting that direct absorption by water

was not the only influence of moss water content on reflectance 970 nm. The maximum

water content values experienced by *Sphagnum* were much greater than those of

*Pleurozium* but they did share a range of common water contents between about 1 and 14

(fresh weight / dry weight). Nonetheless, based on laboratory results, WBI appeared to be

a useful tool to determine species-specific water content without destructive

measurements.

4.1.3 Normalized difference vegetation index and chlorophyll index in the laboratory

As predicted, changes in NDVI were strongly correlated with changes in water

content for water contents at and below optimal values for net photosynthesis. Based on

similar patterns observed between NDVI and CI for *Sphagnum*, the plateau in NDVI at

high water contents was likely not due to saturation of the index caused by high

chlorophyll content. Chlorophyll index, which is less susceptible to saturation at high

chlorophyll content (Gitelson and Merzlyak 1994, Datt 1998), showed the same plateau

for the same range of water content values. The decrease in NDVI and CI observed during dry down, starting near optimal water content for net photosynthesis, agreed with reductions in photosynthetic capacity of mosses observed in drying and re-moistening experiments conducted by Murray et al. (1989) and Schipperges and Rydin (1998). Murray et al. (1989) reported limited recovery of photosynthetic rate, only about 30 and 70% for two *Sphagnum* species one day after rehydration. This suggests that the decline in photosynthesis at low water content was related to reduction in photosynthetic capacity. Low NDVI and CI indicate that this was caused by a reduction in the amount of chlorophyll effectively absorbing visible radiation for photosynthesis. Schipperges and Rydin (1998) also noted poor recovery of *Sphagnum* net photosynthetic rate following complete desiccation and observed bleaching of branch tips similar to observations made of *Sphagnum* in this study. Since NDVI and CI for *Sphagnum* displayed more dramatic declines than *Pleurozium*, it is possible that the effectiveness of chlorophyll within *Pleurozium* was less affected by desiccation than *Sphagnum*. Further assessment of NDVI and CI with remoistening experiments may be used to further assess the extent of the reduced effectiveness of chlorophyll. That is, if reductions in NDVI and CI were due to damage to chlorophyll, rehydration would not yield an immediate recovery of NDVI and CI.

## 4.2 Observations contrary to predictions

### 4.2.1 Spectral vegetation indices in the field

Differences between laboratory and field observations of WBI, NDVI, and CI in relation to water content are potentially attributable to several factors, two of which are

discussed here. Firstly, field observations of WBI and water content in similar research (Houston 2004) reported weak relationships between LUE and WBI ($r^2$=0.127). Weak correlations may have been related to differences in response to drying relative to remoistening due to modified cellular structure. Penuelas et al. (1993) noted that reflectance shifts at 970 nm were more pronounced for vascular plant species with reduced cell wall elasticity during drought stress. The lack of correlation between WBI and water content may be related to recent water status history of the ecosystem combined with subtle variability in growth conditions for individual moss samples in the field. Differences in moss internal structure due to subtle variation in microclimatic conditions could also influence NIR reflectance. Moss samples in the field were selected for their similarity in hummock position, species composition, and overall health assessed visually. These differences could also influence the relationship between NDVI, PRI, and CI relative to water content. Future research is proposed in section 4.3.1 that seeks to further investigate this.

Secondly, reliance on ambient illumination conditions also has the potential to introduce complications for data collection and calibration. Spectral reflectance index values can be strongly influenced by changes in sensor and illumination angle. Complications associated with BRDF can affect a variety of remote sensing applications (Milton 1987, Hatchell 1999). Bi-directional reflectance characteristics may be a contributor to the inconsistency between laboratory and field results. Snyder (1998) summarized that modelling BRDF from reflectance measurements assumes that the adjustment of sensor angle can be used as a proxy for adjustment in illumination angle according to the *Helmholtz reciprocity principle* (von Helmholtz 1924). This suggests

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that maintaining the view angle at nadir will not prevent this phenomenon if illumination source angle is variable. Reflectance measurements using a goniometer demonstrated the potential variability of WBI based on view angle and illumination geometry and, therefore, the configuration of sensor and illumination angle has implications for the interpretation of this index. An enclosed moss sample, single artificial light source, and spectroradiometer foreoptic in fixed geometry could be used control for bi-directional reflectance effects in the future. Such accessories, available for leaf level measurements (Gamon and Surfus 1999), should be adaptable for larger targets such as moss surfaces since single moss leaves are too small to measure individually. Moss leaves are often one cell thick and in *Sphagnum* mosses, usually less than 3 mm long (Johnson et al. 1995). It may, however, be of interest to attempt to measure individual branches or stems of mosses. The intent of these accessories is to block all unwanted direct, diffuse, and transmitted radiation within the measurement realm of both the moss and calibration panel. This should resolve temporally inconsistent illumination conditions (e.g.: variable sun angle), differences in geometry between calibration and target measurement possibly related to uneven moss surfaces, and dynamic shadow patterns cast by overstorey vegetation particularly during windy conditions. Even using this closed system approach, variability between measurements is possible if azimuth-dependent mechanisms influence moss canopy texture. For example, the alignment of moss stems or cellular and intercellular morphology may be influenced by the position of the moss relative to the sun. If this is the case, these factors may be correctable by aligning the sampling device appropriately for each sample. This strategy could involve repetitive measurements of the same targets across the growing season.

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4.2.2 Photochemical reflectance index

Observed changes in PRI relative to water content did not agree with expected

results based on the linkage of PRI to LUE empirically investigated during the

development of PRI (Gamon et al. 1992). Certain methodological concerns can be

explained in the context of the consistent relationships observed between net

photosynthesis and moss water content, agreement between the two spectroradiometers

(Appendix A), and agreement with previous studies of the effects of moss water content

on spectral response curves. Several concerns remain including the speed of reflectance

shifts at 531 nm in relation to xanthophyll interconversion; the possibility that

characteristics of the xanthophyll cycle vary between bryophytes and vascular plants;

potential complications associated with measuring subtle changes in reflectance at narrow

bandwidths while other factors, such as water content, are simultaneously influencing

reflectance; and variation of PRI and photoprotective xanthophyll concentrations over

short and long time scales. These concerns are initiated with a discussion of the effect of

ambient $CO_2$ concentration on net photosynthesis and reflectance.

In the laboratory, following net photosynthesis measurements, the <30 second

time gap between removal of the moss cuvette from the gas exchange chamber and the

reflectance measurement may have allowed for significant xanthophyll shifts to occur

(Demmig Adams 1998). High ambient $CO_2$ concentration in the laboratory air outside the

gas exchange chamber may have increased the $CO_2$ gradient from outside to inside the

moss decreasing the effectiveness of water as a barrier to $CO_2$ diffusion as demonstrated

by Silvola (1990). At high water contents, a rise in intercellular $CO_2$ concentration ($c_i$)

during reflectance measurements could have been significant enough to cause PRI to

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rapidly rise to levels observed at mid-range water content. Three points are presented that may be useful for analyzing the possibility that this and other factors were confounding the expected relationship between PRI and water content.

Firstly, Silvola (1990) demonstrated that ambient $CO_2$ concentration would need to be greater than 900 ppm for net photosynthesis rate of mosses at high water content (8-18 fresh weight / dry weight) to rise to the same rate observed at optimal water contents (6-8 fresh weight / dry weight). Ambient $CO_2$ concentration was not measured in the laboratory during these experiments although it is likely that room $CO_2$ concentration was less than 600 ppm (see Future research section 4.3.2).

Secondly, while experiments conducted by Gamon et al. (1990) noted changes in reflectance at 531 nm within 30 seconds of changing condition, the magnitude of this shift was only about one quarter the shift observed 2 minutes later and about one fifth of that observed within 5 minutes. The shift in reflectance at 531 nm was slow relative to shifts at 685 and 738 nm (Gamon et al. 1990). These shifts in reflectance were in response to the sudden exposure of dark-adjusted leaves to high PPFD, not changes in $CO_2$ concentration (Gamon et al. 1990). It is possible that the rate of xanthophyll conversion differs between dark adjusted plants suddenly exposed to high light and plants adjusted to high light subjected to environmental stresses (as in this thesis). That is, plants adjusted to darkness (night) may have a more sluggish xanthophyll response (minutes) relative to plants adjusted to high light. As reviewed in the introduction, xanthophyll conversions can occur at different rates with different conditions, particularly when converting from Z back to V for shade acclimated plants (Demmig Adams 1998). Future experiments could be conducted where the moss is progressively subjected to high stress

from a low stress condition. The dry down experiments conducted here initially subjected the mosses to stressed conditions ($CO_2$ limitation), followed by optimal conditions, and finally high stress conditions by the end of the experiment.

Thirdly, Houston (2004) observed weak inverse correlations between PRI and water content of Arctic mosses, which was contrary to expected positive relationships based on previous studies of vascular plants (Gamon et al. 1997, Nichol et al. 2000). Houston (2004) found PRI to be weakly and inversely correlated with moss gross photosynthesis rate ($r^2 = 0.33$). This observation of a negative slope was opposite that expected. It was concluded that more research was required to determine if PRI responds to xanthophyll pigment concentrations in bryophytes as it does in vascular plants (Houston 2004). Lovelock and Robinson (2002) found a negative relationship between PRI and $Z$ for Antarctic mosses, as expected, but the relationship was weak ($r^2=0.298$). They also found PRI to be correlated with microtopographic effects and moss water content. The inconsistency in findings between studies of PRI and moss water content and the modest strength (at best) of relationships emphasizes the significant influence of unknown factors on moss PRI. To successfully interpret PRI for mosses, more must be learned about the variables that influence it, including the plant's water status history (see Future research section 4.3.1).

If PRI is to be used to it's full potential, the influence of all significant factors on reflectance of 531 and 570 nm must be understood. The body of literature on PRI can be used to provide insights as to what factors contribute to a dynamic response in different scenarios. Many studies report weak relationships between PRI and LUE. For example, a coefficient of determination 0.38 was observed in a study of PRI and LUE of

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Mediterranean trees (Penuelas et al. 1997). Penuelas et al. (1997) attributed the poor

relationship to two factors. One factor was canopy architecture changes due to leaf

wilting during drought stress conditions. The other was the disassociation of photosystem

II efficiency from leaf net photosynthesis because of leaf processes competing with $CO_2$

uptake for chemical energy produced by photosystem II. In vascular plants, Carter (1991)

showed that the effect of absorption of radiation by water increased with longer

wavelengths from visible to near infrared radiation. The secondary effect of water

content, the subtle shifts in 531 nm reflectance due to changes in the ratio of $Z$ to $V$, could

be masked by the overriding primary effect of water absorption in mosses subject to a

wide range of water contents. The PRI normalization band at 570 nm may be more

influenced by water content than the 531 nm band. If this is the case, it may explain why

3 of the 5 *Sphagnum* replicates were not correlated with LUE, and why the other 2 of the

5 *Sphagnum* replicates were directly correlated with water content. Gamon et al. (1992)

noted that while PRI was correlated with diurnal LUE shifts for sunflower canopies

subject to nitrogen stress, PRI was not correlated with water stressed canopies

undergoing severe wilting. This may be due to the over-riding primary effects of water

content on leaf reflectance. As an additional consideration, it is possible that the effects of

desiccation may have inhibited the function and/or interconversion of xanthophyll

pigments.

It is possible that PRI is more sensitive to noise caused by subtle errors in

measurement, instrumentation, and/or calibration that do not appear to greatly influence

NDVI and WBI (Appendix A). Increased susceptibility to noise may be related to two

factors. Firstly, there is a very small difference in reflectance observed at 531 nm

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between leaves with very high and leaves with very low Z concentrations (Gamon et al. 1990). As a result, the range of 531 nm reflectance values, as indicators of Z concentration in leaves, are susceptible to these errors. This is because the increment of change in reflectance at 531 nm representing a significant change in Z is small relative to increments of change in reflectance at 531 nm caused by errors. Relative to PRI, the differences in reflectance are higher at 670 nm between leaves with very high and very low chlorophyll content, or reflectance at 970 nm between leaves with very high and very low water content. Based upon observations of the spectral response curves, the appearance of what seems to be uncertainty in reflectance values due to noise or radiometric resolution error can be as high as 0.0005 (as a proportion of reflectance) for wavelengths in the visible portion of the spectrum. For example, consider this reflectance uncertainty of approximately +/-0.0005 relative to a decrease in reflectance at 531 nm of 0.005, as observed for sunflowers following exposure to high irradiance (Gamon et al. 1990). In this scenario, about +/-10% uncertainty is introduced into this interpretation that there has been a major shift in Z concentration. If the same error were applied to WBI where a shift in reflectance at 970 nm of 0.15 indicated a major change in water content for *Sphagnum* (Figure 6), only +/-0.3% uncertainty is introduced. Secondly, plants reflect only a small proportion of incident visible radiation. The assignment of PRI to this portion of the spectrum elevates the susceptibility of not just one, but both bandwidths (531 and 570 nm) to low signal to noise ratios. With the radiometric sensitivity limitations of current spectroradiometer technology, radiometric resolution errors may reduce the ability to universally estimate xanthophyll concentrations from PRI values.

Potential exists to use PRI to compare relative xanthophyll pool sizes between

mosses adapted and/or acclimated to different environmental conditions. Photosynthesis

of *Pleurozium* (shade plant) saturates at lower light intensity compared to *Sphagnum*. In

the field, PRI values measured for *Pleurozium* were higher than PRI values measured for

*Sphagnum* (Figure 15). This result was in agreement with observations of low $Z$

concentrations relative to chlorophyll in shade plants (Demmig-Adams 1998). However,

the laboratory results did not agree with this because the PRI values of *Pleurozium* were

lower than those of *Sphagnum* (Figure 11). The PRI values of both species in the

laboratory were substantially higher than in the field (Figure 11). This may be due to the

fact that these mosses were maintained in growth chambers at low light for a period of

about two months. The mosses may have responded to this by reducing $Z$ and carotenoid

pool sizes relative to chlorophyll content. It is possible that the response of *Sphagnum*

was more pronounced than that of *Pleurozium*. Perhaps any short-term shifts in PRI due

to diurnal environmental variation were smaller and therefore more difficult to detect

relative to seasonal acclimation shifts of total xanthophyll concentrations. The usefulness

of PRI as a tool to interpret photochemical efficiency will improve with greater

understanding of the effects of diurnal and seasonal variation in environmental controls

on vegetation function and reflectance.


## 4.3 Future research

Suggestions for future research are described in this section. The first suggestion

involves studying how moss spectral reflectance responds to re-moistening after exposure

to dry-down. To further investigate the response of PRI relative to net photosynthesis

rate, the second suggestion involves varying moss net photosynthesis rate by changing

atmospheric $CO_2$ instead of water content. The final section offers two further options for

pursuing knowledge about moss reflectance and physiology.

4.3.1 Moss re-moistening experiments

This thesis research examined the potential impact of bi-directional reflectance as

one of the causes of the discrepancy between lab and field results, but the influence of re-

moistening on spectral vegetation indices was not explored. It is possible that re-

moistening would result in a different relationship between moss water content and

NDVI, CI, and WBI relative to that observed during the dry down experiment. Murray et

al. (1989) showed that net photosynthesis rates of *Sphagnum angustifolium* and

*Sphagnum squarrosum* were lower after dry-down and re-moistening than they were

before dry-down. It is possible that the WBI is influenced not only by direct absorption

by water but is also influenced by the recent water status history of the plant.

These proposed experiments would investigate the nature of the response of

spectral vegetation indices to the recent water status history of the plant. Specifically, the

proposed objective would be to test how WBI responds to water content of mosses with

varied water content histories. Also, this experiment would reveal how quickly NDVI and

CI (effectively absorbing chlorophyll content) recover following reductions in water

content at specified rates and magnitudes of change in water content.

When the mosses are rehydrated from water contents below optimal for net

photosynthesis, NDVI and CI should either show no response or delayed increases (lag

effect). Delayed increases would indicate recovery of visible radiation absorption by

chlorophyll. Alternatively, if NDVI and CI are influenced strongly by absorption by

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water, they will be correlated with water content as the moss is rehydrated from low water contents. If WBI is influenced primarily by absorption by water, it is expected that WBI will respond linearly with water content, consistent with the linear equations found in the thesis for each species (Figure 12). If WBI index does not respond as expected, it is likely that reflectance at 970 nm is more influenced by secondary effects of water content on the leaf than by direct absorption by water. Other water absorption bandwidths such as the 1200, 1500, and 2000 nm middle infrared bands are measurable when using the ASD-FR FieldSpec but are out of the spectral range of the more portable Unispec spectroradiometer.

A concern when comparing the laboratory results to the field is that the removal of the capitula from basal portion of the moss would increase desiccation effects and hinder recovery of photosynthesis. This is because *Sphagnum* mosses use capillarity and dense growth in hummocks to enhance drought tolerance (Schipperges and Rydin 1998). The relationship between spectral vegetation indices and water content could be further investigated using dry down experiments with the mosses remaining in the large moss bins used to maintain the mosses in growth chambers or in the field over consistent locations. Since spectral measurements need to be made over consistent locations to test the relationship between WBI and water content, moss clippings for water content measurements would need to be collected from adjacent locations. This could introduce error if water content at adjacent locations is not identical to water content under the spectroradiometer foreoptic. The effectiveness of determining water content by measuring electrical conductivity in the moss, using a soil moisture probe or other device, could be tested against the oven drying technique.

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4.3.2 Varying photochemical reflectance and moss net photosynthesis rate by changing atmospheric $CO_2$ concentration

This proposed experiment would further examine the response of PRI in mosses to changes in high light stress. During laboratory experiments, mosses underwent large variations in water content that may have influenced PRI and masked the indirect reflectance effects of changes in $Z$ concentration. It is also possible that no changes in $Z$ concentration occurred as indicated by PRI (Figure 11). At high water content, carbon fixation by mosses is limited by $CO_2$ availability (Silvola 1990). Associated with this limitation, $Z$ concentrations should be high and PRI should therefore be low. If ambient $CO_2$ concentration was increased progressively, $CO_2$ would become less limiting to photosynthesis since the diffusion gradient between the air and the chloroplast would increase. Increased supply of $CO_2$ would result in increased demand by photosynthesis for photon-derived energy. This would reduce the need for high $Z$ concentrations.

A further step may involve eliminating the possible influence of exposure to ambient $CO_2$ during the time required to move the moss from the chamber to the spectroradiometer stage. This could involve developing a $CO_2$ exchange chamber that can accommodate a spectroradiometer foreoptic. In this case, considerations such as placement of the foreoptic to avoid shadowing or variable diffusion of incoming irradiance must be considered. Also, if the light source for spectral measurements were external to the glass chamber, the radiation attenuation effects of the chamber would need to be accounted for.

If PRI responds as predicted to changes in $CO_2$ concentration, this would be evidence for a functional xanthophyll cycle in mosses. In the context of the thesis experiments, it would also suggest that PRI is not an effective tool for estimating shifts in moss photochemical efficiency caused by large changes in water content.

### 4.3.3 Additional perspectives on future research

Intercomparison of ground level, tower-based, and satellite-based reflectance data collected at the same time for the same feature can validate the future use of reflectance data collected from various scales. Sequentially evaluating reflectance measurements from ground level, as in this thesis, followed by coarse ground level (canopy scale), high spatial resolution airborne, high resolution satellite, moderate resolution satellite, and finally coarse resolution satellite measurements, respectively, would enable a valuable progressive evaluation of the scaling effects of spectral reflectance indices of the Western Peatland. One satellite data source to consider building into this comparison is the moderate resolution imaging spectrometer (MODIS). This sensor collects imagery of Earth's entire surface every day and data from this sensor is freely available. A seasonal comparison of field reflectance data at ground level, canopy level and high altitude with flux tower data could be used to compare and contrast large-scale subtleties to small-scale generalizations.

Much of remote sensing science is focused on measuring reflectance from high altitudes so as to acquire information quickly over large land areas. To consider the utility of given spectral vegetation indices for a specified application at various scales it is useful to consider the major biological factors that could cause changes in reflectance

with changes in sensor altitude. To conceptualize the problem it may be useful to temporarily ignore some common complications such as atmospheric variability, sensor noise, and bi-directional effects (e.g.: Milton and Goetz 1997). In addition, to consider that these factors can likely be corrected for and possibly even incorporated to verify or acquire specific biological information (e.g.: canopy structure). For mosses, the utility of an index that measures the effective absorption by a pigment (e.g.: NDVI measuring absorption by chlorophyll) might not be expected to change a great deal with altitude/pixel size. One could consider that the moss surface (as used in the lab and field measurements in this thesis) resembles a 'forest' with many trees branches. The measurements in this thesis were essentially recorded from above the moss canopy that was conveniently comprised of species pure stands with minimal debris/multiple species/soil background effects. In this scenario, NDVI was not a useful indicator of LAI or biomass because biomass and LAI remained constant throughout the laboratory experiments. This is because the samples were 'optically thick' (i.e.: covering the underlying material such that no radiation can penetrate the vegetation and be reflected back to the sensor) from beginning to end of the experiment. The indirect link between NDVI to LAI and biomass was broken in the thesis laboratory experiment because changes in absorption by chlorophyll were not related to changes in biomass and LAI (these remained constant) but rather due to down regulation of the chlorophyll molecules themselves. The scaling up of NDVI as a measure of biochemical capacity for the moss 'canopy' to a higher altitude does not seem to pose as great a challenge as for other vegetative life forms (e.g.: large trees and shrubs) for which canopy measurements in the laboratory and field are far less feasible. In such a case, leaf measurements are required

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as well as accounting for branches, trunks, and often-sporadic openings in canopy revealing the many other endmembers that require accounting for in field spectroradiometer measurements. It appears that one of the very difficult factors in scaling up with mosses would be accounting for the many different overstorey species that might completely cover a large portion of the underlying moss 'canopy'.

A final suggestion is to consider using spectral mixture analysis (Peddle et al. 2001b) to acquire information about plant function at the leaf level. Instead of using selected portions of the optical spectrum, as with spectral vegetation indices, spectral mixture analysis incorporates the entire optical reflectance spectra of as many pure components as desired. This would involve obtaining spectral reflectance of pure pigments, then applying these 'libraries' via spectral mixture analysis to attempt to estimate xanthophyll concentrations. These suggested topics for future study are posed to assist advances in remote sensing techniques to improve our understanding of terrestrial carbon cycling.

One current limitation to current biological methods such as gas flux measurements is the ability to measure large land areas efficiently. With appropriate distribution of equipment, tracking of weather conditions, knowledge of ecosystem distribution, and even the use of taller eddy covariance towers, ecosystem flux models can likely be quite accurate at predicting carbon fluxes. In addition to ecosystem flux models, remote sensing also offers a means of overcoming this limitation assuming that appropriate and accurate biological information can be derived from reflectance data from high altitudes.

# 5 CONCLUSIONS

From the empirical assessments conducted in this research, steps have been taken toward using spectral vegetation indices to understand moss characteristics related to net photosynthesis rates. The main objective of this study was to investigate the utility of spectral vegetation indices for deriving information about factors influencing net photosynthetic rate (i.e. biochemical capacity and photochemical efficiency) of two boreal peatland mosses: *Pleurozium* and *Sphagnum*. Varying moss water content over the short term in the laboratory enabled evaluation of changes in NDVI, CI, PRI, and WBI in relation to changes in net photosynthesis. Ground-based field studies of moss reflectance attempted to test these indices in the field. Laboratory reflectance measurements using a goniometer helped examine the susceptibility of indices to variable configurations of illumination and sensor geometry. In doing so, it is apparent that bi-directional reflectance effects must be considered when further developing repeatable ground based measurement.

This thesis research has contributed to knowledge in the fields of Geography and Biology. Contributions to Geography included an empirical assessment of how remote sensing science can provide information about photosynthetic capacity and efficiency of mosses as varied by water content. The thesis provided empirical evidence for the potential of the selected reflectance indices to provide non-destructive remotely sensed information about moss dominated ecosystem characteristics that strongly influence carbon cycling. It also further developed the prospect of using multi-angular reflectance measurements to extract biological information, and suggested some multi-scale

approaches to obtaining more detailed vegetation information. Such developments can serve to fuel future research such as the building of radiative transfer models for mosses including specific carotenoid pigments.

Contributions to Biology included a unique way of observing the morphological and physiological differences between *Pleurozium* and *Sphagnum* mosses adapted for different hydrologic environments. Specifically, these differences were found to be observable via non-destructive remotely acquired spectral indices. This research did not produce evidence for an active xanthophyll cycle in mosses from which future research methods for further testing were suggested.

Ultimately, this thesis has made a contribution to climate change research by enhancing methods to more efficiently and non-destructively acquire information about moss dominated ecosystems. Understanding how these carbon rich environments respond to environmental change will enable future improvements to climate change forecasting and in turn, influence how people manage factors influencing environmental change.

The five most important final conclusions that can be made based on this research project are as follows. Firstly, moss net photosynthesis rate and spectral reflectance were strongly influenced by moss water content. Secondly, reduced NDVI at low water content supported the view that a decline in $CO_2$ uptake was related to reduced biochemical capacity in *Sphagnum*. Thirdly, PRI was different between species but did not vary with large reductions in moss water content and variability in $CO_2$ uptake. These results suggest that as visible reflectance varies with moss water content due to absorption by water, subtle variations in reflectance at 531 nm due to changing xanthophyll pigment concentrations may be difficult to distinguish. As a result, PRI may not be viable for

directly estimating light use efficiency in mosses if light use efficiency varies due to changes in moss water content. Fourthly, WBI was strongly linearly related to water content under controlled laboratory conditions but not in the field. Fifthly, goniometer measurements demonstrated that large (1.24 – 1.40) variations in WBI for a single location on the moss surface could be associated with view angle. The potential to estimate moss water content from WBI will support further testing of field techniques to address potential complicating factors and to improve this method.

Current approaches for acquiring information about the responses of global ecosystems to changing climatic variables involve leaf and canopy scale ecophysiological measurements extrapolated over large land cover classes. Remote sensing of ecosystem spectral reflectance including satellite-derived information also offers a means to efficiently acquire information related to ecosystem $CO_2$ exchange across large areas. Remote sensing methods depend upon an understanding of leaf and canopy reflectance response to environmental factors. Additionally, measurement of high spectral resolution leaf reflectance may be used as a ground level remote sensing tool to help explain mechanistic reasons for changes in net $CO_2$ uptake. These endeavours may succeed in enabling the use of reflectance measurements as a tool for acquiring biological information such as moss water content. This would enable direct calculation of net photosynthesis rates using parameter driven models of moss physiology (Williams and Flanagan, 1998). Calculated photosynthesis rates can be used to understand the effects of environmental changes on net $CO_2$ exchange and to efficiently quantify the contribution of mosses to net ecosystem exchange. This research can also lead to enhanced interpretation of ground-level or canopy scale reflectance as collected using tram systems

or high altitude sensors. This can be done by defining key linkages between spectral and physiological characteristics of mosses as one of several components of peatland landscapes. Application of this knowledge to current, future and historical remotely sensed data across broad spatial scales will improve our ability to quantitatively predict how the fluxes of these high carbon stock ecosystems will respond to climate change. Terrestrial carbon cycling research has tremendous potential to advance via the efficient, repeatable, and non-destructive biophysical parameter acquisition capabilities of remote sensing.

# REFERENCES

Adams WW, Zarter CR, Ebbert V, and Demmig-Adams B (2004) Photoprotective
strategies of overwintering evergreens. *BioScience* 54: 41-49

Ahrens CD (2000) *Meteorology Today: an introduction to weather, climate, and the
environment*. Brooks/Cole Pacific Grove, CA, 528 p.

Baret F and Guyot G (1991) Potentials and limits of vegetation indices for LAI and
APAR assessment. *Remote Sensing of Environment* 35: 161-173

Belyea LR and Malmer N (2004) Carbon sequestration in peatlands: patterns and
mechanisms of response to climate change. *Global Change Biology* 10: 1043-
1053

Bilger W, Bjorkman O, and Thayer S (1989) Light-induced spectral absorbance changes
in relation to photosynthesis and the epoxidation state of xanthophyll cycle
components in cotton leaves. *Plant Physiology* 91: 542-551

Blankenship RE (2001) *Molecular Mechanisms of Photosynthesis*. Blackwell Science
Oxford, UK, 328 p.

Bubier JL, Rock BN, and Crill PM (1997) Spectral reflectance measurements of boreal
wetland and forest mosses. *Journal of Geophysical Research* 102: 29,483-29,494

Carter GA (1991) Primary and secondary effects of water content on the spectral
reflectance of leaves. *American Journal of Botany* 78: 916-924

Chapin FS, Matson PA, and Mooney HA (2002) *Principles of terrestrial ecosystem
ecology*. Springer-Verlag New York, 436 p.

Chen JM (1996) Evaluation of vegetation indices and a modified simple ratio for boreal
applications. *Canadian Journal of Remote Sensing* 22: 229-242

Clymo RS and Hayward PM (1982) The ecology of *Sphagnum*. In *Bryophyte Biology*,
edited by A.J.E. Smith, Chapman and Hall, New York, 1982, pp 229-290

Coburn CA and Peddle DR (2005). A low-cost field and laboratory goniometer system
for estimating hyperspectral bi-directional reflectance of vegetation canopies.
Proceedings, 26th Canadian Symposium on Remote Sensing. Wolfville, Nova
Scotia, June 14-16, 10 p.

Crippen RE (1990) Calculating the vegetation index faster. *Remote sensing of
Environment* 34: 71-73

Datt B (1998) Remote sensing of chlorophyll a, chlorophyll b, chlorophyll a + b, and
total carotenoid content in eucalyptus leaves. *Remote Sensing of Environment* 66:
111-121

Demmig B, Winter K, Kruger A, and Czygan FC (1988) Zeaxanthin and the heat
dissipation of excess light energy in *Nerium oleander* exposed to a combination of
high light and water stress. *Plant Physiology* 87: 17-24

Demmig-Adams B and Adams WW (1996a) The role of xanthophyll cycle carotenoids in
the protection of photosynthesis. *Trends in Plant Science* 1: 21-26

Demmig-Adams B and Adams WW (1996b) Xanthophyll cycle and light stress in nature:
uniform response to excess direct sunlight among higher plant species. *Planta*
198: 460-470

Demmig-Adams B (1998) Survey of thermal energy dissipation and pigment composition
in sun and shade leaves. *Plant Cell Physiology* 39: 474-482

87

Demmig-Adams B, Moeller DL, Logan BA, and Adams WW (1998) Positive correlation between levels of retained zeaxanthin + antheraxanthin and degree of photoinhibition in shade leaves of *Schefflera arboricola* (Hayata) Merrill. *Planta* 205: 367-374

Fernandes RH, White HP, Peddle DR, Miller JR, and Bond LE (1996) The reflectance of *Pluerozium schreberi* as a function of water status and its implications on undercanopy reflectance variations for BOREAS sites. *International Geoscience and Remote Sensing Symposium (IGARSS '96)*. Lincoln, NB 1: 212-214

Field CB, Ball TB, and Berry JA (1989) Photosynthesis: principles and field techniques. In *Plant Physiological Ecology*, edited by Pearcy RW, Ehleringer JR, Mooney HA, and Rundel PW, Chapman and Hall, New York pp 209-254

Frolking S, Roulet NT, Moore TR, Lafleur PM, Bubier JL, and Crill PM (2002) Modeling seasonal to annual carbon balance of Mer Bleue Bog, Ontario, Canada. *Global Biogeochemical Cycles* 16: 2001GB001457

Gamon JA, Field CB, Bilger W, Bjorkman O, Fredeen AL, and Penuelas J (1990) Remote sensing of the xanthophyll cycle and chlorophyll fluorescence in sunflower leaves and canopies. *Oecologia* 85: 1-7

Gamon JA, Penuelas J, and Field CB (1992) A narrow-waveband spectral index that tracks diurnal changes in photosynthetic efficiency. *Remote Sensing of Environment* 41: 35-44

Gamon JA, Filella I, and Penuelas J (1993) The dynamic 531-nanometer Δ reflectance signal: a survey of twenty angiosperm species. *Current Topics in Plant Physiology* 8: 172-177

Gamon JA, Serrano L, and Surfus JS (1997) The photochemical reflectance index: an optical indicator of photosynthetic radiation use efficiency across species, functional types, and nutrient level. *Oecologia* 112: 492-501

Gamon JA and Surfus JS (1999) Assessing leaf pigment content and activity with a reflectometer. *New Phytologist* 143: 105-117

Gausman HW (1985) *Plant leaf optical properties in visible and near-infrared light* Graduate Studies Texas Tech University Texas 78 p.

Gitelson AA and Merzlyak MN (1994) Spectral reflectance changes associated with autumn senescence of *Aesculus hippocastanum* L. and *Acer platanoides* L. leaves. Spectral features and relation to chlorophyll estimation. *Journal of Plant Physiology* 143: 286-292

Gitelson AA, Merzlyak MN, and Lichtenthaler HK (1996) Detection of red edge position and chlorophyll content by reflectance measurements near 700 nm. *Journal of Plant Physiology* 148: 501-508

Gorham E (1991) Northern peatlands: role in the carbon cycle and probable responses to climatic warming. *Ecological Applications* 1: 182-195

Haboudane D, Miller JR, Pattey E, Zarco-Tejada PJ, Strachan IB (2004) Hyperspectral vegetation indices and novel algorithms for predicting green LAI of crop canopies: modeling and validation in the context of precision agriculture. *Remote Sensing of Environment* 90: 337-352

Hall FG, Peddle DR, and Ledrew EF (1996) Remote sensing of biophysical variables in boreal forest stands of *Picea mariana. International Journal of Remote Sensing* 17: 3077-3081

88

Harley PC, Tenhunen JD, Murray KJ, and Beyers J (1989) Irradiance and temperature effects on photosynthesis of tussock tundra *Sphagnum* mosses from the foothills of the Philip Smith Mountains, Alaska. *Oecologia* 79: 251-259

Hatchell DC (1999) Analytical Spectral Devices Inc. (ASD) Technical Guide, Analytical Spectral Devices, Boulder, CO 136 p.

Hilbert DW, Roulet N, and Moore T (2000) Modelling and analysis of peatlands as dynamical systems. *Journal of Ecology* 88: 230-242

Holt NE, Zigmantas D, Valkunas L, Li XP, Niyogi KK, and Fleming GR (2005) Carotenoid cation formation and the regulation of photosynthetic light harvesting. *Science* 307: 433-436

Houston S (2004) *Separating arctic tundra vegetation types based on photosynthetic light-use efficiency and optical properties,* Master's Thesis, California State University, LA, 103 p.

Johnson D, Kershaw L, MacKinnon A, Pojar J, Goward T, and Vitt D (1995) *Plants of the western boreal forest and aspen parkland* Lone Pine Publishing, Edmonton, Alberta 392 p.

IPCC (2001) *International Panel on Climate Change. Climate change 2001: the scientific basis. Contribution of working group I to the third assessment report* Cambridge University Press, Cambridge, England, 944 p.

Lambers H, Chapin III FS, and Pons TL (1998) *Plant Physiological Ecology,* Springer-Verlag New York, 540 p.

Lovelock CE and Robinson SA (2002) Surface reflectance properties of Antarctic moss and their relationship to plant species, pigment composition, and photosynthetic function. *Plant, Cell, and Environment* 25: 1239–1250

Milton EJ (1987) Principles of field spectroscopy. *International Journal of Remote Sensing* 8: 1807-1827

Milton EJ, and Goetz AFH (1997) Atmospheric influences on field spectrometry: observed relationships between spectral irradiance and the variance in spectral reflectance. *7th International symposium on physical measurements and signatures in remote sensing (ISBN)* Balkema, Netherlands 90: 109-114

Murray KJ, Harley PC, Beyers J, Walz H, and Tenhunen JD (1989) Water content effects on photosynthetic response of *Sphagnum* mosses from the foothills of the Philip Smith Mountains, Alaska. *Oecologia* 79: 244-250

Myneni RB and Williams DL (1994) On the relationship between FAPAR and NDVI. *Remote Sensing of Environment* 49: 200-221

Nichol CJ, Huemmrich KF, Black TA, Jarvis PG, Walthall CL, Grace J, and Hall FG (2000) Remote sensing of photosynthetic-light-use efficiency of boreal forest. *Agricultural and Forest Meteorology* 101: 131-142

Nicodemus FE, Richmond JC, Hsia JJ, Ginsberg IW, and Limperis T (1977) *Geometrical considerations and nomenclature for reflectance*. National Bureau of Standards, U.S. Department of Commerce Washington, D.C. 52 p.

Painter TH, Paden B, and Dozier J (2003) Automated spectro-goniometer: a spherical robot for the field measurement of the directional reflectance of snow. *Review of Scientific Instruments* 74: 5179-5188

Peddle DR, Hall FG, LeDrew EF (1999). Spectral mixture analysis and geometric-optical reflectance modeling of boreal forest biophysical structure. *Remote Ssensing of Eenvironment* 67: 288-297.

Peddle DR, White HP, Soffer RJ, Miller JR, and LeDrew EF (2001a) Reflectance processing of remote sensing spectroradiometer data. *Computers & Geosciences* 27: 203-213

Peddle DR, Brunke SP, and Hall FG (2001b) A comparison of spectral mixture analysis and ten vegetation indices for estimating Boreal forest biophysical information from airborne data. *Canadian Journal of Remote Sensing* 27: 627-635

Peddle DR, Johnson RL, Cihlar J, Latifovic R (2004) Large area forest classification and biophysical parameter estimation using the 5-Scale canopy reflectance model in Multiple-Forward-Mode. *Remote Sensing of Environment* 89: 252-263

Penuelas J, Filella I, Biel C, Serrano L, and Save R (1993) The reflectance at the 950-970 nm region as an indicator of plant water status. *International Journal of Remote Sensing* 14: 1887-1905

Penuelas J, Llusia J, Pinol J, and Filella I (1997) Photochemical reflectance index and leaf photosynthetic radiation-use-efficiency assessment in Mediterranean trees. *International Journal of Remote Sensing* 18: 2863-2868

Penuelas J and Inoue Y (1999) Reflectance indices indicative of changes in water and pigment contents of peanut and wheat leaves. *Photosynthetica* 36: 355-360

Penuelas J, and Inoue Y (2000) Reflectance assessment of canopy $CO_2$ uptake. *International Journal of Remote Sensing* 21: 3353-3356

Proctor MCF (2000) Physiological ecology. In *Bryophyte Biology*, edited by Shaw AJ, Cambridge University Press, New York, pp 225-247

Rahman AF, Gamon JA, Fuentes DA, Roberts DA, and Prentiss D (2001) Modeling spatially distributed ecosystem flux of boreal forest using hyperspectral indices from AVIRIS imagery. *Journal of Geophysical Research* 106: 33,579-33,591

Rouse JJW, Haas RH, Schell JA, and Deering DW (1973) Monitoring vegetation systems in the Great Plains with ERTS. In *Proc. Earth Res. Tech. Satellite-1 Symp* Goddard Space Flight Centre Washington, DC pp 309–317

Schaaf CB, Gao F, Strahler AH, Lucht W, Li X, Tsang T, Strugnell NC, Zhang X, Jin Y, Muller J-P, Lewis P, Barnsley M, Hobson P, Disney M, Roberts G, Dunderdale M, Doll C, d'Entremont RP, Hu B, Liang S, Privette JL, and Roy D (2002) First operational BRDF, albedo nadir reflectance products from MODIS. *Remote Sensing of Environment* 83: 135-148

Schipperges B and Rydin H (1998) Response of photosynthesis of *Sphagnum* species from contrasting microhabitats to tissue water content and repeated desiccation. *New Phytologist* 140: 677-684

Sellers PJ, Berry JA, Collatz GJ, Field CB, and Hall FG (1992) Canopy reflectance, photosynthesis, and transpiration. III. A reanalysis using improved leaf models and a new canopy integration scheme. *Remote Sensing of Environment* 42: 187-216

Silvola J (1990) Combined effects of varying water content and $CO_2$ concentration on photosynthesis in *Sphagnum fuscum. Holarctic Ecology* 13: 224-228

Sims DA and Gamon JA (2003) Estimation of vegetation water content and photosynthetic tissue area from spectral reflectance: a comparison of indices

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based on liquid water and chlorophyll absorption features. *Remote Sensing of Environment* 84: 526-537

Snyder WC (1998) Reciprocity of the bidirectional reflectance distribution function (BRDF) in measurements and models of structured surfaces. In *IEEE Transactions on Geoscience and Remote Sensing* pp 685-691

SPOT (2004) Systeme pour l'observation de la Terre Website: http://www.spotimage.fr/html/_167_.php. Accessed on June 6, 2005.

Stylinski CD, Gamon JA, and Oechel WC (2002) Seasonal patterns of reflectance indices, carotenoid pigments, and photosynthesis of evergreen chaparral species. *Oecologia* 131: 366-374

Taiz L and Zeiger E (2002) *Plant Physiology*. Sinauer Associates, Inc. Sunderland, MA, 690 p.

Tol RSJ (2005) The marginal damage costs of carbon dioxide emissions: an assessment of the uncertainties. *Energy Policy* 33: 2064-2074

Tonn, B (2003) An equity first, risk-based framework for managing global climate change. *Global Environmental Change* 13: 295-306

Turner M (1998) The Sandmeier field goniometer: a measurement tool for bi-directional reflectance. *NASA Commercial Remote Sensing Verification and Validation Symposium* NASA Ames Research Center, John C. Stennis Space Center, MS 6 p.

Turunen JE, Tolonen TK, and Reinikainen A (2002) Estimating carbon accumulation rates of undrained mires in Finland – applications to boreal and subarctic regions. *Holocene* 12: 69-80

United Nations Framework Convention on Climate Change (2005) Website: http://unfccc.int/2860.php. Accessed on June 6, 2005

Vanacker V, Linderman M, Lupo F, Flasse S, and Lambin E (2005) Impact of short-term rainfall fluctuation on interannual land cover change in sub-Saharan Africa. *Global Ecology & Biogeography* 14: 123-135

Vogelmann JE and Moss DM (1993) Spectral reflectance measurements in the genus *Sphagnum*. *Remote Sensing of Environment* 45: 273-279

von Helmholtz H (1924) *Helmholtz's Treatise on Physiological Optics*. Dover Publications, Inc. New York, NY 479 p.

Williams TG and Flanagan LB (1996) Effect of changes in water content on photosynthesis, transpiration, and discrimination against $^{13}CO_2$ and $C^{18}O^{16}O$ in *Pleurozium* and *Sphagnum*. *Oecologia* 108: 38-46

Williams TG and Flanagan LB (1998) Measuring and modelling environmental influences on photosynthetic gas exchange in *Sphagnum* and *Pleurozium*. *Plant, Cell, and Environment* 21: 555-564

Zarco-Tejada PJ, Berjon A, Miller JR (2004) Stress detection in crops with hyperspectral remote sensing and physical simulation models. *Proceedings of the Airborne imaging spectroscopy workshop - Bruges*. 8 October 2004 5 p.

# APPENDIX A: COMPARISON OF ASD-FR FIELDSPEC AND UNISPEC SPECTRORADIOMETERS

## Introduction

Instrumentation has the potential to add uncertainty to experimental results. Differing capabilities of spectroradiometers such as spectral and radiometric resolution as well as imperfections in factory calibration may interfere with absolute reflectance values. This thesis research was afforded the luxury of using two spectroradiometers for reflectance measurements. The use of both in simultaneous measurements of the same targets enabled a high degree of confidence in the quality of spectral data from laboratory experiments with mosses. It also offered the potential to compare these instruments to measure average reflectance of spectrally 'stable' objects. Since the instruments have different resolutions and were calibrated independently, it was of interest to investigate the agreement between the two. It was expected that while dissimilarities in measurements of reflectance would exist, these differences would not be great enough to significantly alter the interpretation of biophysical information provided by spectral response curves and spectral vegetation indices.

## Methods

Spectral reflectance data were acquired using two spectroradiometers (Figure 17) at the University of Lethbridge: a Unispec Spectral Analysis System (PP Systems, Haverhill, MA) courtesy of the laboratory of L.B. Flanagan and an Analytical Spectral Devices FieldSpec Full Range (ASD-FR) spectroradiometer (ASD, Inc. Boulder, CO) courtesy of the Spectroradiometer Laboratory of D.R. Peddle. Instrument resolutions, settings, and reflectance measurement procedures for the moss dry down experiment are described in the methods section in the thesis, chapter 2.3 and further noted in Table 9.

Two data sets were used to compare the agreement between the spectroradiometers. The first was the reflectance data collected during the moss dry down experiment in the laboratory. The second was reflectance data of 20 x 20 cm square book covers each with constant colour across their surface (blue paper, green paper, purple paper, white paper, and red vinyl). The selection of distinctly coloured objects enhanced the comparison by showing the relative abilities of the instruments to respond to dramatic shifts at various locations of the visible spectrum.

Each spectroradiometer recorded ten measurements of each target (except for red vinyl, n=5) utilizing the apparatus shown in Figure 3. The procedure for each reflectance measurement involved a radiance measurement of a reference panel (25 cm X 25 cm spectralon) followed by a radiance measurement of the object. A 500 W halogen lamp illuminated the targets from a consistent angle from nadir (45°) for all measurements. Foreoptics of both instruments were placed at nadir orientation 20 cm above the target.

Table 9: Spectroradiometer parameters for reflectance measurement comparison between 400 and 1000 nm.

| Parameter | ASD-FR FieldSpec | Unispec |
|---|---|---|
| Spectral resolution (FWHM) | 3 nm | 10 nm |
| Sampling integration time | 136 ms | 50 ms |
| Angular field of view | 18 degrees | 28 degrees |
| Height above target | 20 cm | 20 cm |
| Field of view diameter | 6.3 cm | 10 cm |
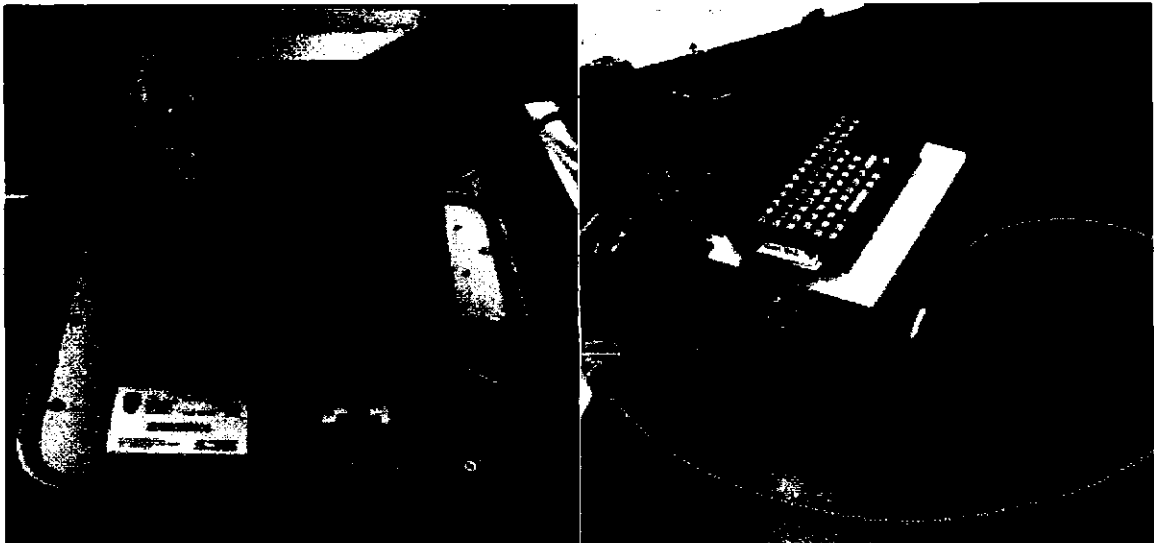


Figure 17: Photographs of the ASD-FR FieldSpec (left) and Unispec (right) spectroradiometers.

**Results and Discussion**

Agreement of spectral vegetation index responses recorded by the two spectroradiometers can be observed in the application of these instruments to the moss dry down experiments (Figure 18). Linear regression line slopes of the WBI versus water content relationship produced using both instruments for *Sphagnum* were not statistically different (t=1.836, df=128, p>0.05) for water contents less than 22 (fresh weight / dry weight). Values higher than 22 were eliminated because WBI began to saturate above this water content. While regression slopes of WBI versus water content for *Pleurozium* had statistically significantly different regression line slopes between spectroradiometers (t=4.6361, df=150, p<0.05), the line slopes provide for a close interpretation considering that the instruments have different levels of sophistication and separate manufacturers. There was a greater degree of dissimilarity between the PRI versus water content relationship relative to that of WBI, however the variability of PRI values within individual instrument measurements during the experiments was about the same as the variability between instruments (Figures 19 and 20). This indicated that the differences between instruments were only a moderate concern for the interpretation of PRI. The index with the greatest difference between spectroradiometers was PRI while WBI showed the least difference as is further illustrated in the coefficient of determination values in Figures 19 and 20. These graphs illustrate the sensitivity of PRI to instrumentation characteristics that did not appear to greatly influence NDVI, CI, and WBI. This may be related to the fact that differences in reflectance are greater between variable and reference wavebands of NDVI, CI, and WBI relative to PRI. Since both wavebands used for calculating PRI have relatively low reflectance values, they may be more strongly influenced by the ability of the instruments to record an accurate signal relative to background noise.

Spectral response curves recorded for the various spectrally stable materials illustrate the effective range of the Unispec between 400 and 1000 nm (Table 10 and Figures 21 to 25). The data at the extreme ends of the Unispec output (<400 & >1000 nm) did not appear suitable for use. The spectroradiometers produced spectral response curves with a close match for these materials (Figures 21 to 25) and the greatest amount of deviation can be seen for the purple paper (Figure 23), where the Unispec yielded slightly lower visible reflectance and slightly higher NIR reflectance relative to the ASD-FR FieldSpec. The absolute difference in reflectance between the two spectroradiometers for measurements of red vinyl was low (Table 10 and Figure 26). For red vinyl, the mean difference between spectroradiometers (400 – 1000 nm) was 0.6% with a standard error of 0.03%. The greatest difference in reflectance was 1.82% at 611 nm, the portion of the spectrum where the greatest change in reflectance occurred due to the red colour of the material. The reason for this difference between the two spectroradiometers at 611 nm may have been due to the differences in spectral resolution of the instruments. The change in reflectance between 600 and 620 nm of the red book cover was so great that minor shifts in spectral sampling would yield different results. The ASD-FR FieldSpec more accurately characterized this portion of the spectrum because it closely recorded the large reflectance changes per wavelength rather than averaging across broader bandwidths.

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The similarities between instruments shown here and information from the literature suggest that they are performing well and that generalized relationships between spectral and biophysical data are comparable across instruments. The differences observed suggest that precise best-fit equations developed for spectral/biophysical relationships using one instrument should not be directly applied to another instrument. Reflectance data acquired by one instrument might not be universally comparable to reflectance acquired by the other instrument. In spite this, the small differences observed between reflectance of the two instruments were likely not great enough to significantly alter the interpretation of information provided by spectral response curves and spectral vegetation indices in this thesis.

Table 10: Descriptive statistics comparing the reflectance difference between 3 nm wavebands of red vinyl spectral response curves measured by Unispec and ASD-FR FieldSpec spectroradiometers (ASD minus Unispec). These statistics are presented for the full spectral range of the Unispec (350 – 1137 nm) and for the usable range (400 – 1000 nm). Five scans of the red vinyl were recorded and averaged by each spectroradiometer prior to calculating absolute differences at each waveband.

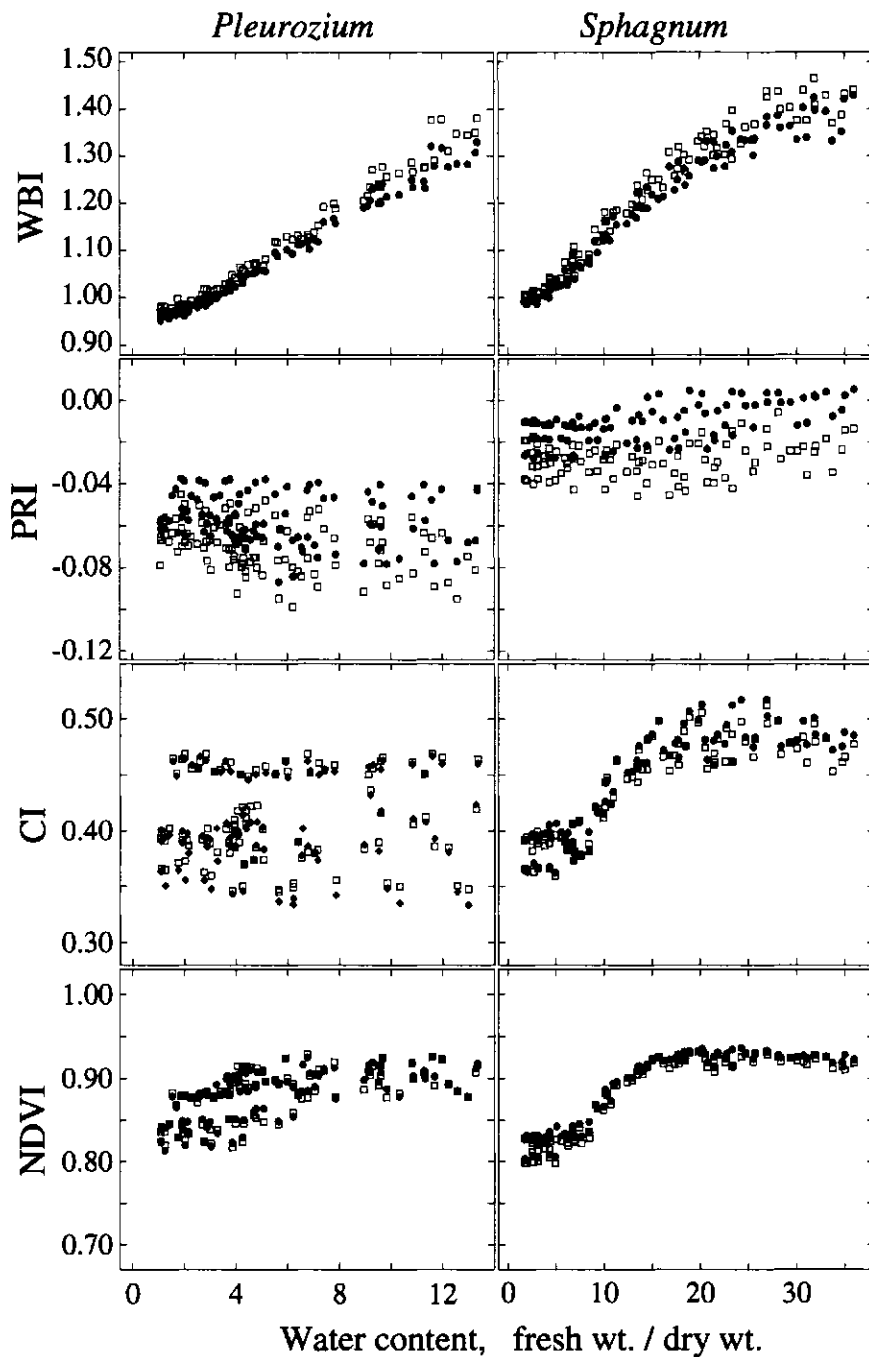|  | ASD reflectance minus Unispec reflectance from 350 to 1137 nm | ASD reflectance minus Unispec reflectance from 400 to 1000 nm |
|---|---|---|
| mean | 0.0283 | 0.0060 |
| median | 0.0066 | 0.0061 |
| stdev | 0.0640 | 0.0036 |
| SE | 0.0041 | 0.0003 |
| min | 0.0004 | 0.0004 |
| max | 0.3321 | 0.0182 |
| n | 242 | 185 |

Figure 18: Influence of changes in moss water content on four spectral vegetation indices in the laboratory measured with two spectroradiometers. Symbols represent two sets of spectral measurements collected in tandem by Unispec □ and ASD-FR FieldSpec ● spectroradiometers during dry down of a set of 5 moss replicates.
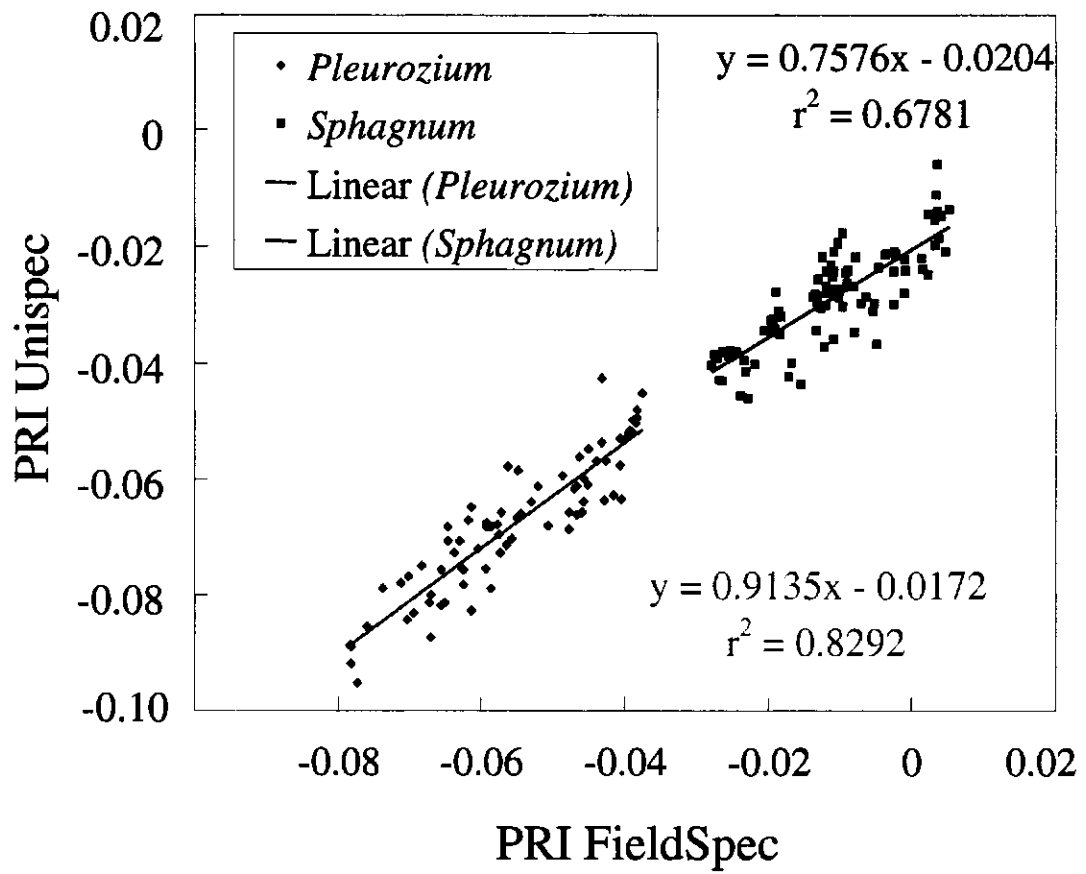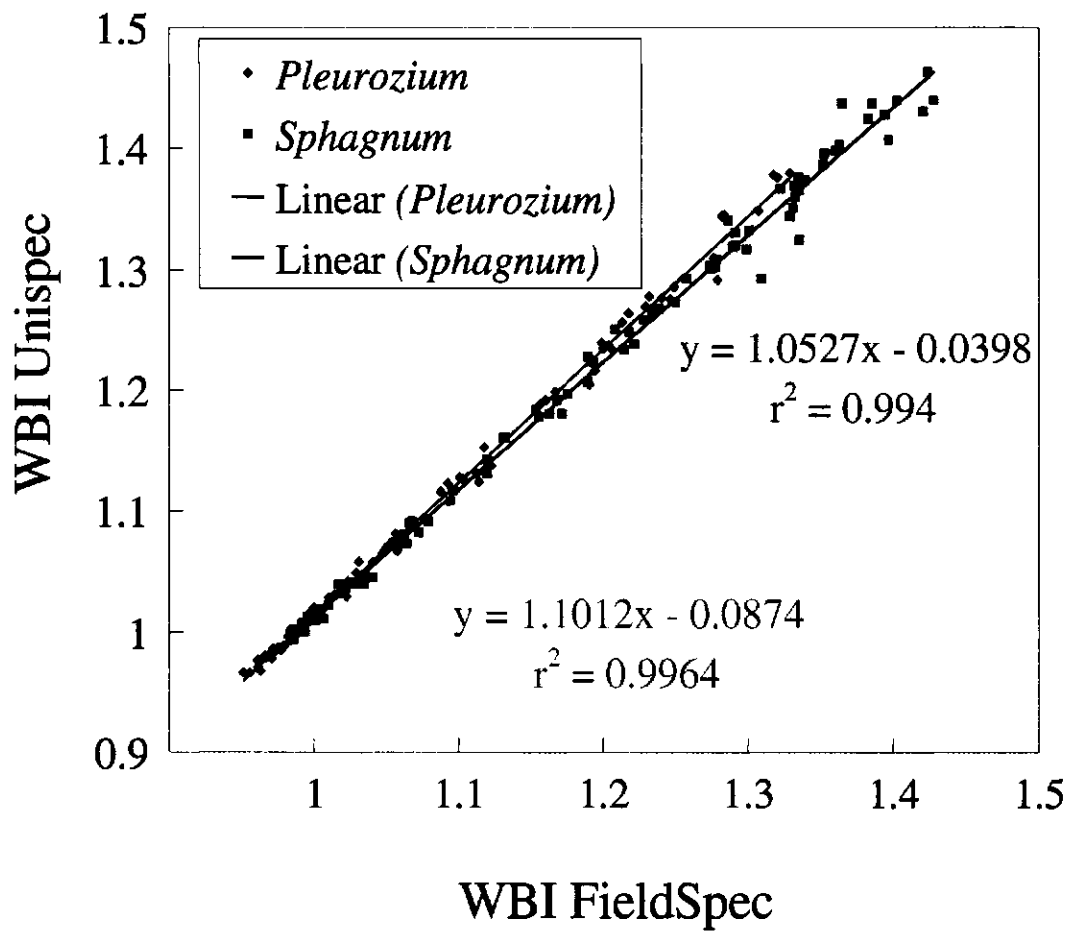
Figure 19: Comparison of Unispec and ASD-FR FieldSpec spectroradiometer PRI measurements of *Pleurozium* and *Sphagnum* measured during laboratory dry down experiments.
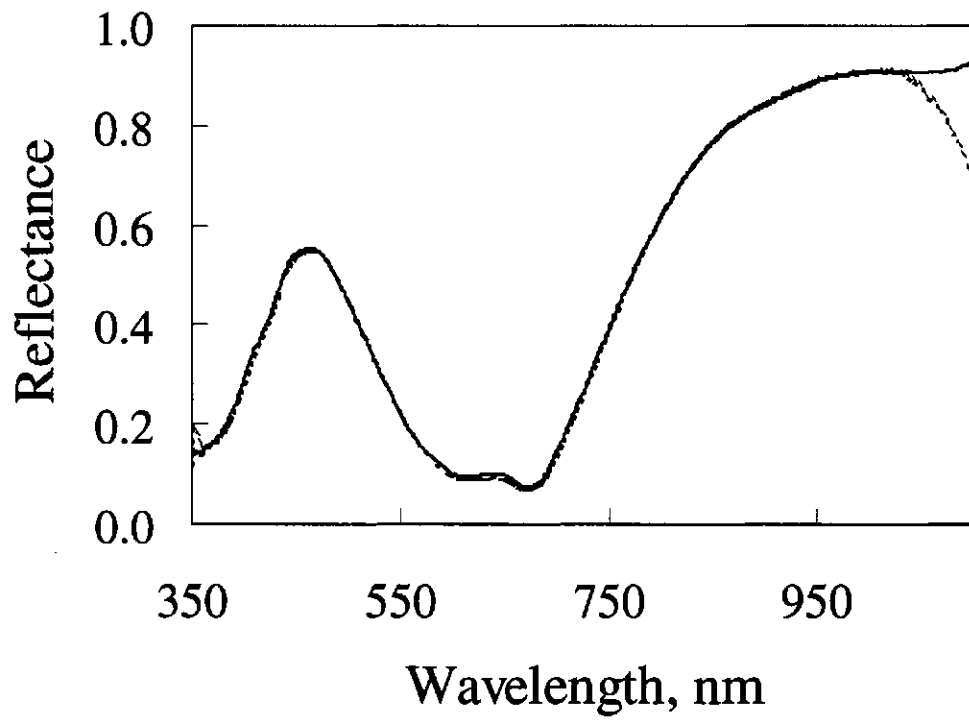
Figure 20: Comparison of Unispec and ASD-FR FieldSpec spectroradiometer WBI measurements of *Pleurozium* and *Sphagnum* measured during laboratory dry down experiments.
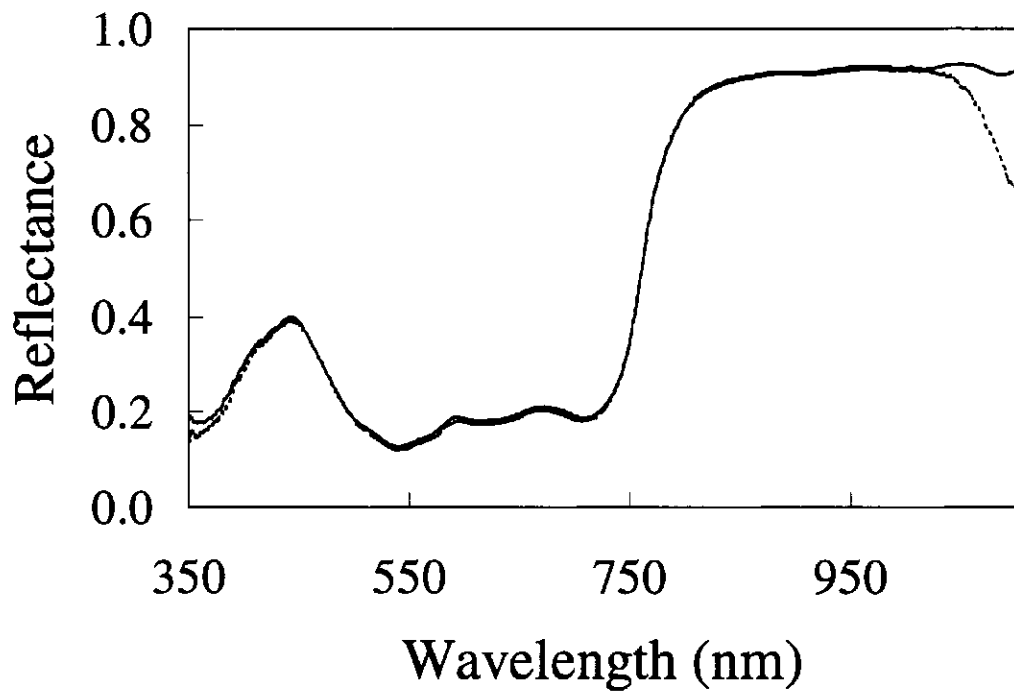
Figure 21: Spectral response curves of blue paper collected using Unispec ········· and ASD-FR FieldSpec ———— spectroradiometers. Lines represent mean reflectance values (n=10).
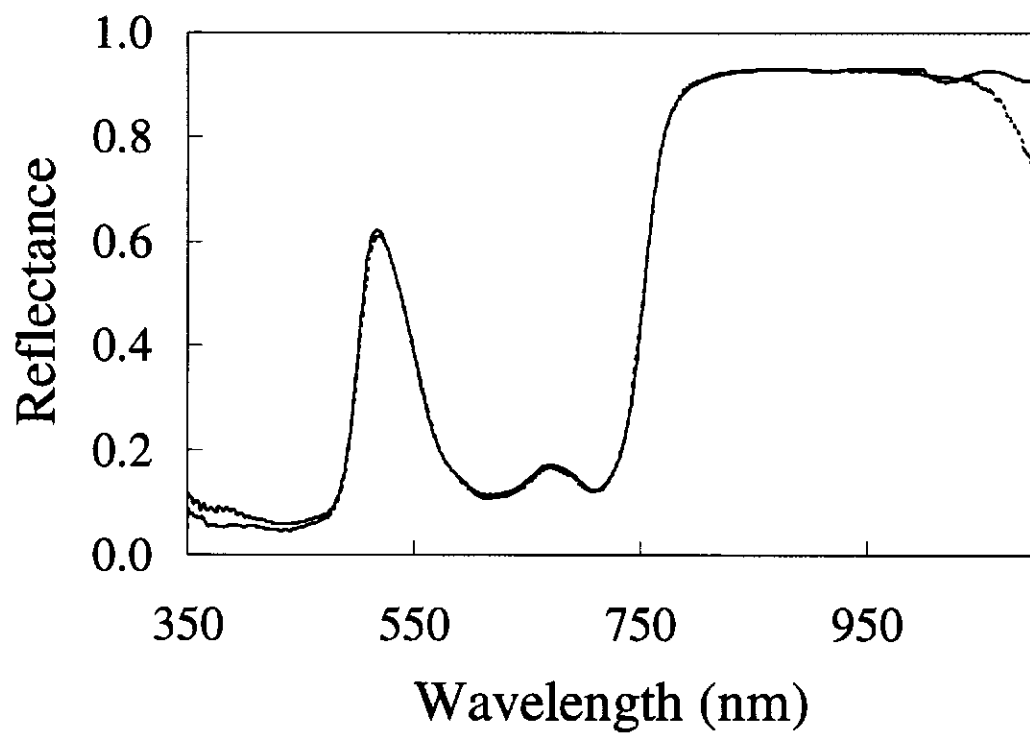
Figure 22: Spectral response curves of green paper collected using Unispec ‑‑‑‑‑‑‑ and ASD-FR FieldSpec ——— spectroradiometers. Lines represent mean reflectance values (n=10).

Figure 23: Spectral response curves of purple paper collected using Unispec ········ and ASD-FR FieldSpec ———— spectroradiometers. Lines represent mean reflectance values (n=10).
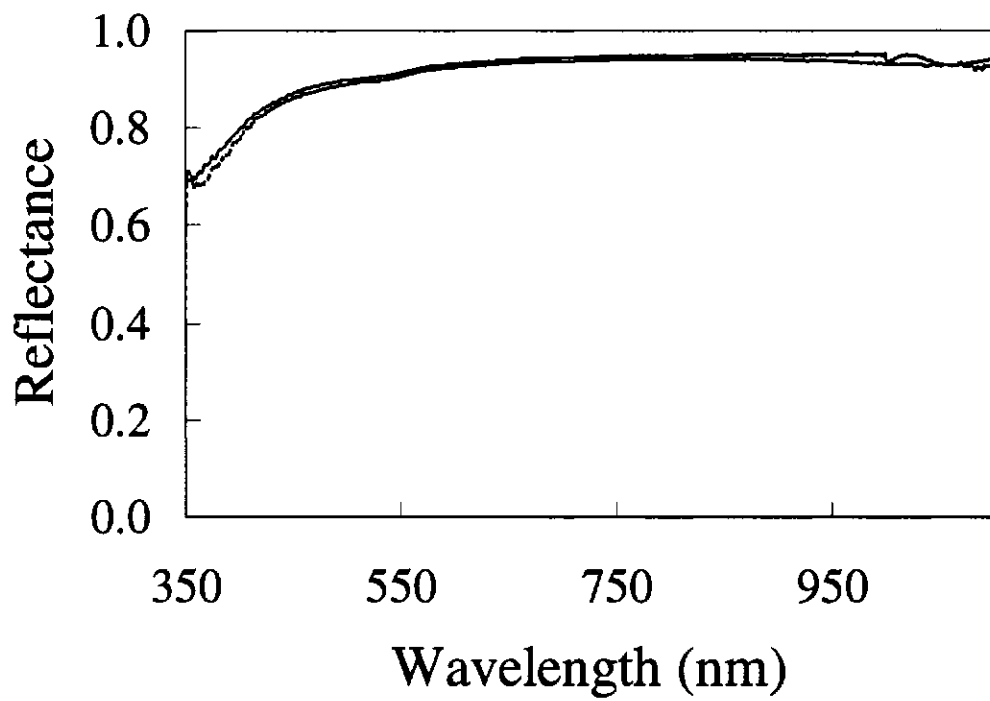
Figure 24: Spectral response curves of white paper collected using Unispec ········ and ASD-FR FieldSpec ——— spectroradiometers. Lines represent mean reflectance values (n=10).
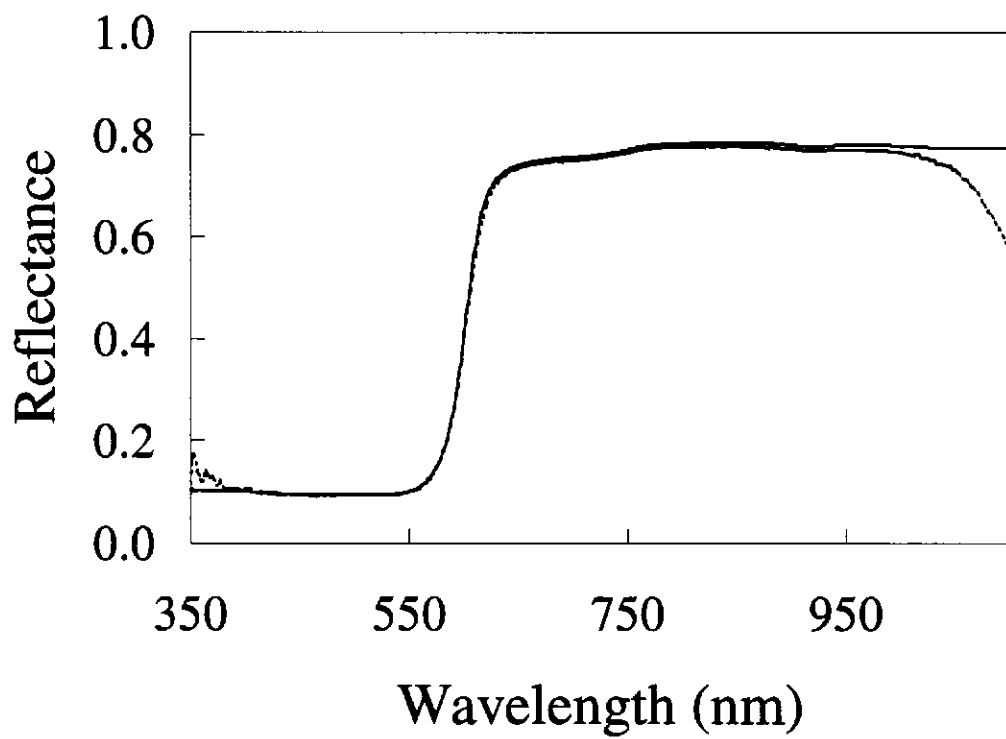
Figure 25: Spectral response curves of red vinyl collected using Unispec ------- and ASD-FR FieldSpec ———— spectroradiometers. Lines represent mean reflectance values (n=5).
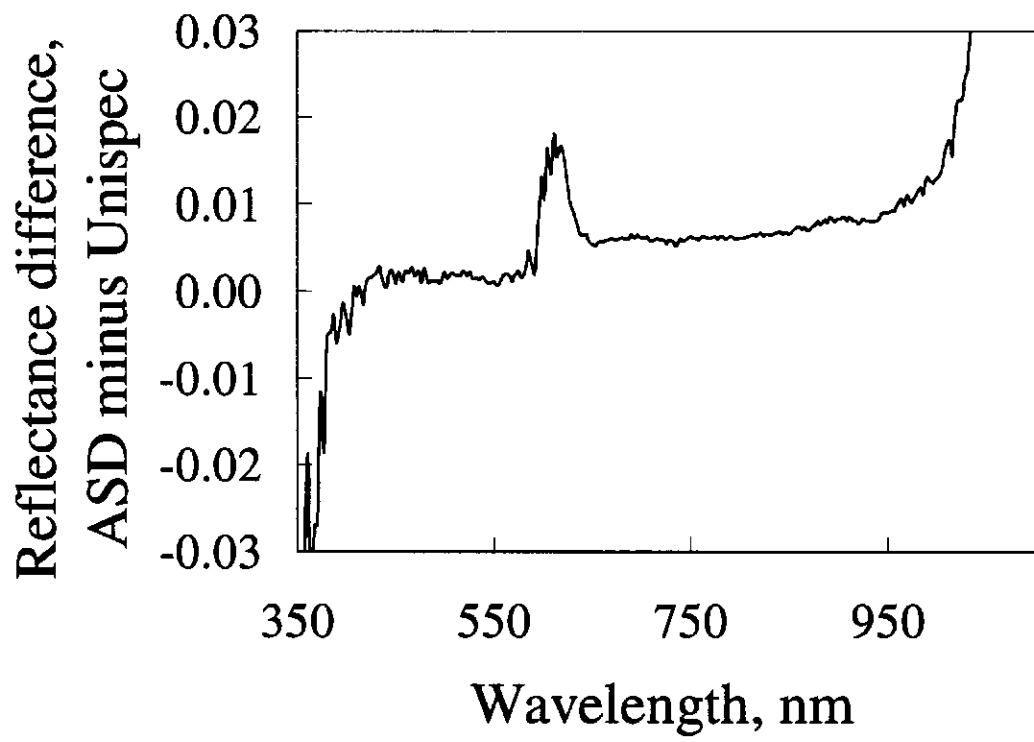
Figure 26: Difference between Unispec and ASD-FR FieldSpec spectroradiometer mean reflectance measurements of red vinyl with magnified y-axis scale.

# APPENDIX B: ATTEMPTS TO CONTROL ILLUMINATION FOR REFLECTANCE MEASUREMENTS OF MOSSES

## Introduction

Reliance on ambient illumination conditions introduces complications for data collection and processing logistics. Atmospheric variability, bi-directional reflectance effects, and variability in shadowing and diffuse lighting effects are such complications. These can be especially problematic under a forest canopy and when it is windy. Some of these could be resolved by using an artificial light source within an enclosure to block ambient radiation from reaching the moss surface. The method described here is called the Enclosed Artificial Radiation (EAR) technique. This technique would provide more consistent illumination conditions between the calibration panel and target measurement. The purpose of this work was to develop, test, and improve controlled illumination of small moss targets in the field. It primarily addresses the ability of radiation to transmit through the moss canopy.

## Methods

Reflectance measurements were attempted in the field using enclosed artificial illumination (EAR) to the moss surface. This approach used a bifurcated fibre optic cable (UNI410 mini glass, PP systems, Haverhill, MA) as described by Gamon and Surfus (1999). The cable was comprised of randomly bundled fibre optics that branched apart at one end of the cable. One of these branches was connected to a 7 W internal halogen light source contained within the Unispec to deliver irradiation to illuminate the target. The other branch transmitted radiation reflected by the target back to the spectroradiometer. The end of the bifurcated cable was outfitted with an accessory foreoptic attachment (black plastic cylindrical film canister) for covering 2 cm diameter patches of moss to create a closed illumination system (Figure 27). This was intended to block ambient radiation and only allow radiation from the 7 W internal halogen light to strike the target. The foreoptic was suspended within the cylinder 2.4 cm above the moss surface yielding an FOV of 1.2 cm at the moss surface.

Measurements of moss water content and moss reflectance using EAR were made during six field campaigns between May and September 2004. Also, to investigate differences observed between field and laboratory data, a test was conducted to measure how effective the cylinder accessory was at shielding ambient radiation. It was thought that even though the surface of the moss was completely covered by the canister, the surrounding moss was not. It was conceivable that solar radiation was transmitting into the moss surface outside the light shield through the moss 'canopy', and into the field of view of the spectroradiometer. The test involved the use of EAR in the laboratory to measure the surface reflectance of *Pleurozium* and *Sphagnum* growing in large moss bins. The moss bins were subjected to one of two illumination intensities while measuring reflectance. The first was 600 μmol PPFD $m^{-2}$ $s^{-1}$ from a 500 W halogen lamp (45° zenith, 30 cm from moss bin) and the second was ambient laboratory illumination of

15 $\mu$mol photons m$^{-2}$ s$^{-1}$. Ten radiance measurements were recorded under each illumination condition. An additional set of measurements was collected, using a cylindrical tube (12 cm diameter) surrounding the light-shielding canister and counter sunk approximately 3 cm below the moss surface to block radiation travelling through the moss canopy.



Figure 27: Photographs of the Enclosed Artificial Radiation Technique components. Shown in the left photo are the bifurcated fibre optic probe (1a), the bifurcated fibre optic cable (1b), the light shielding canister accessory that attaches onto the probe (2), and the additional 12 cm diameter tube for countersinking into the moss surface to block radiation transmitting through the moss canopy (3). The right photo shows the whole apparatus put together and being used in the field with the Unispec in the foreground.

## Results and Discussion

Water band index measured in the field using EAR demonstrated weak correlation with a wide range of water contents (Figure 28). This contrasted with laboratory dry down experiments using conventional reflectance measurement techniques (Figure 12). The EAR technique used in the field during the summer of 2004 provided intensity values for comparison of approximate ratio vegetation index values with water content throughout the growing season (Figures 29 and 30) but required further improvement to acquire reliable measures of absolute reflectance as will be described. Some of the data, collected in sunflecks, yielded unusual spectral response curves with sometimes extremely high and occasionally saturating reflectance. This effect was repeated in the laboratory test (Figures 31 and 32). The normalization involved in the calculation of some of the indices may have reduced the impact of the addition of

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ambient radiation transmitting through the moss canopy into the field of view. Ambient radiation transmitted through the moss canopy had a strong influence on intensity readings. Transmission was particularly high in the NIR portion of the spectrum relative to the visible. It was also noted that when ambient illumination is almost completely eliminated, particularly when using the larger cylinder counter-sunk into the moss, the reflectance was quite low across the spectrum. Although the standard vegetation spectral response with high NIR reflectance and low visible reflectance was clearly recognizable, concern was raised about the foreoptic being too far (2.4 cm) from the moss surface to provide a substantial reflectance return back to the sensor due to the decay rate of radiation intensity with distance. The PPFD provided from the tip of the fibre optic cable was 25 $\mu$mol m$^{-2}$ s$^{-1}$ and PPFD 2.4 cm from the tip of the fibre optic cable was 7 $\mu$mol m$^{-2}$ s$^{-1}$. These factors may have been contributing to the inability to recognize the strong positive correlation between WBI and water content as seen in laboratory dry down experiments using a constant and strong illumination source.

Probing these differences also involved examining the bi-directional reflectance properties of the moss (Figure 16). WBI varies based on view angle and illumination geometry. When the zenith angles of the foreoptic and illumination source were equal and their azimuths opposite, hot spots did not exist. The configuration of the sensor and illumination source at this geometry would be a proxy for the configuration of the field apparatus geometry according to studies of BRDF (Snyder 1998) that refer to the *Helmholtz reciprocity principle* (von Helmholtz 1924). Since this is the theoretical approximation of the moss light shield configuration with both foreoptic and illumination source at nadir, bi-directional reflectance does not appear to be a likely cause for the low WBI values seen in the field.

In conclusion, measurements using artificial illumination in the field seemed to provide a coarse measure of relative target radiance but not an effective absolute measure of target reflectance. Complications associated with this approach may be due to a combination of two factors. Firstly, it was possible that artificial illumination provided by the bifurcated cable was too far from the target to provide the signal strength required for resolving fine spectral details. Secondly, based on moss transmittance tests, radiation transmitted more easily through *Sphagnum* canopy than through *Pleurozium* canopy, likely due to differences in density. Calibration measurements of the spectralon disc would not have accounted for this transmitted radiation. As more radiation was permitted to transmit through the canopy, the amount of radiation absorbed at 970 nm relative to 900 nm was more easily detectable. As a result, WBI was still found to be weakly correlated with *Sphagnum* but not *Pleurozium* water content. With further improvements, such as increased artificial illumination intensity and improved blockage of solar radiation, this system may provide a reliable means of acquiring controlled reflectance data in the field thereby reducing complicating BRDF effects, and variability in natural illumination.
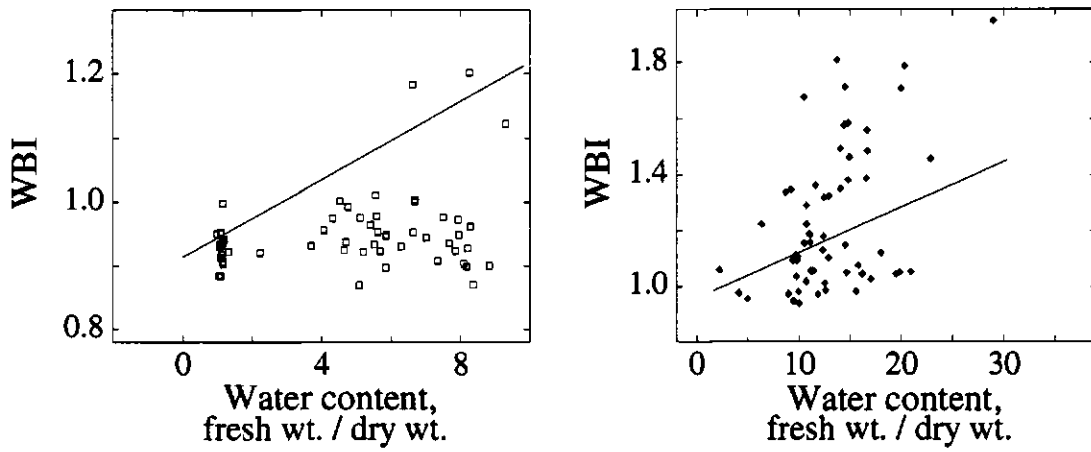
Figure 28: Influence of changes in water content on WBI across season in the field using artificial illumination source for *Pleurozium* (left) and *Sphagnum* (right). The fit lines represent the WBI versus water content relationship found from the laboratory dry down experiments.
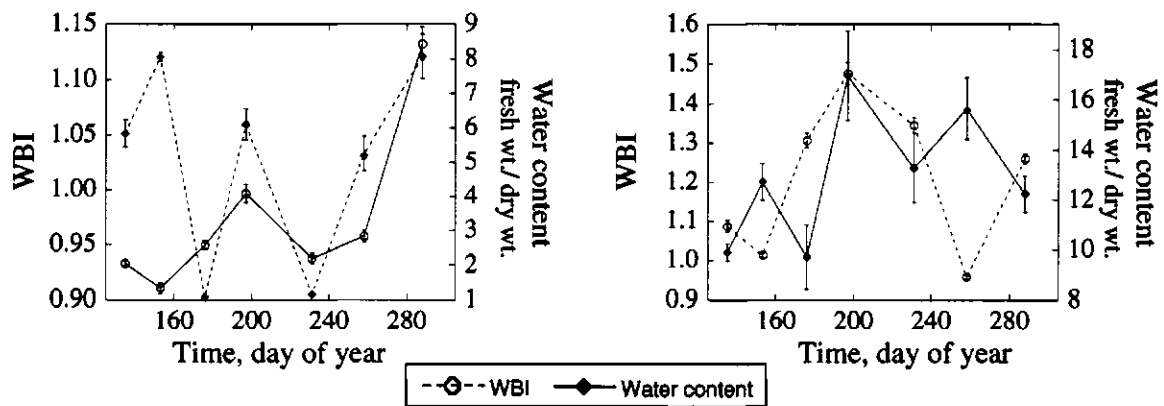


Figure 29: Seasonal variation in WBI and water content for *Pleurozium* (left) and *Sphagnum* (right). Points represent mean values and error bars represent standard error of the mean (for water content, n=10; for WBI n=100 for all measurement days except the last two: n=25 & 10 respectively).
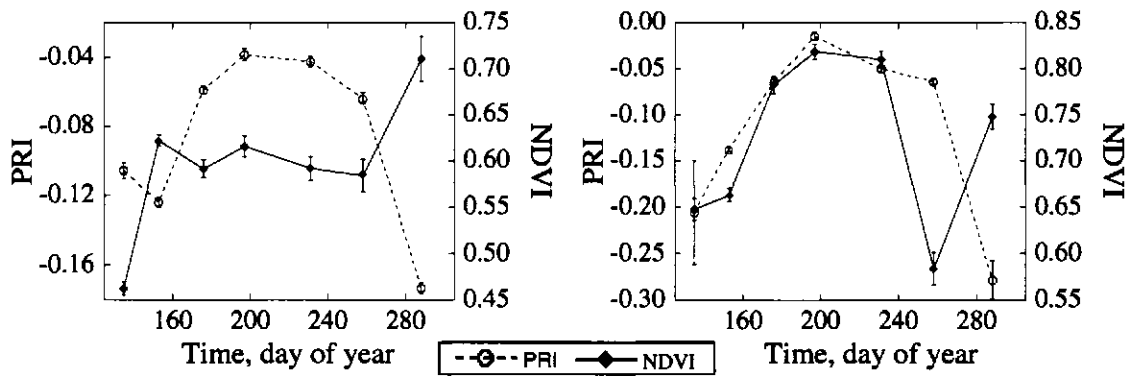
Figure 30: Seasonal variation of PRI and NDVI for *Pleurozium* (left) and *Sphagnum* (right). Points represent mean values and error bars represent standard error of the mean (n=100 for all measurement days except the last two: n=25 & 10 respectively).
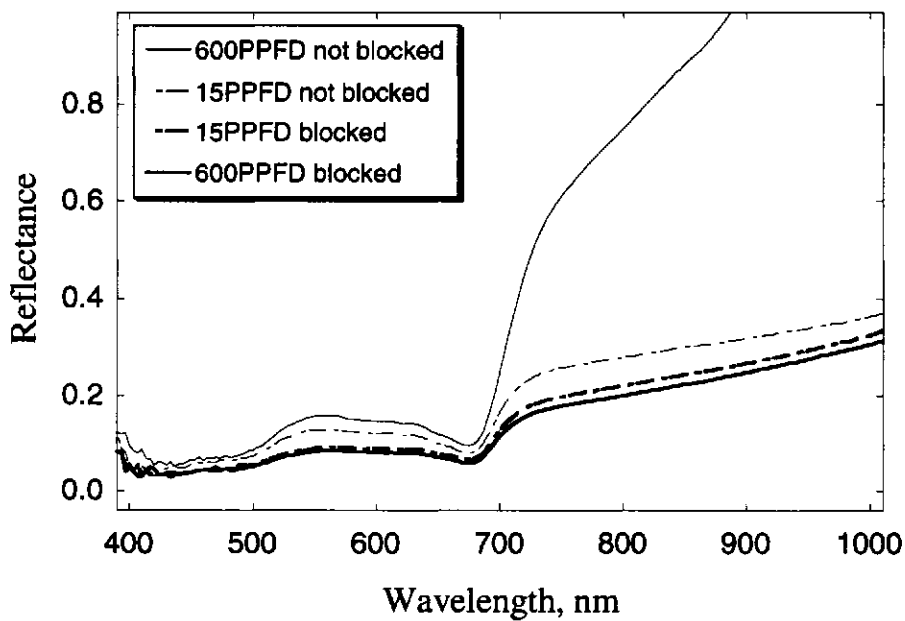


Figure 31: Effect of ambient illumination on attempts to measure visible and near infrared reflectance of *Pleurozium* in a large bin in the laboratory using artificial illumination. Reflectance measured using the moss light shield with and without additional attempts to block radiation transmitting through the moss canopy.
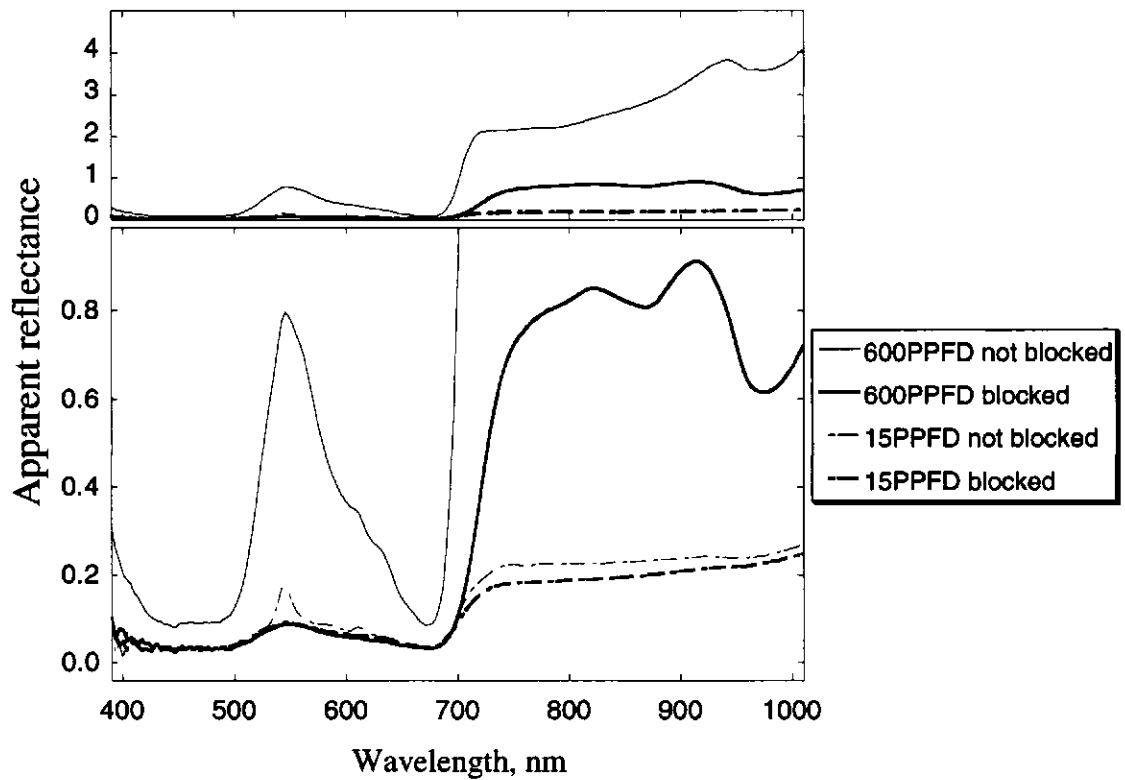
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Figure 32: Effect of ambient illumination on attempts to measure visible and near infrared reflectance of *Sphagnum* in a large bin in the laboratory using artificial illumination. Reflectance measured using the moss light shield with and without additional attempts to block radiation transmitting through the moss canopy.