Hyperspectral Proximal Sensing of the Botanical Composition and Nutrient Content of New Zealand Pastures

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Abstract

The potential of hyperspectral proximal sensing to quantify sward characteristics important in making critical decisions on the management of sheep and dairy pastures in New Zealand has been investigated.

Hyperspectral data were acquired using an ASD FieldSpec® Pro FR spectroradiometer attached to the Canopy Pasture Probe (CAPP). The CAPP was developed to enable the collection of *in situ* reflectance data from New Zealand pasture canopies independent of ambient light conditions. A matt white ceramic tile was selected as a reflectance standard to be used with the CAPP, after testing a variety of materials. Pasture reflectance factor spectra between 350-2500 nm (with spectral resolutions of 3 nm between 350-1000 nm and 10 nm between 1000-2500 nm) and pasture samples were collected from six hill country and lowland areas, across all seasons (August 2006 to September 2007) in a number of regions in the North Island of New Zealand.

After pre-processing (e.g. spectral averaging, de-stepping, elimination of noisy wavelengths, smoothing) the spectral data collected from sites were correlated against pasture botanical composition (expressed as proportions of grass, legume and weed) and pasture nutrients (nitrogen, phosphorus, potassium, calcium, magnesium, sodium and sulphur) expressed in percentage of dry matter (%) and amount (kg ha^{-1}) using partial least squares regressions (PLSR). The accuracy and precision of the calibrations were tested using either the full cross-validation leave-one-out method or testing datasets. Regressions were carried out using the reflectance factor data per se and after mathematical transformation, including first derivative, absorbance and continuum-removed spectra. Overall best results were obtained using the first derivative data. The quality of predictions varied greatly with the pasture attribute, site and season.

Some reasonable results were achieved for the prediction of pasture grass and legume proportions when analysing samples collected during autumn (grass: $R^2 > 0.81$ and SD/RMSEP ≥ 2.3 and legume: $R^2 > 0.80$ and SD/RMSEP ≥ 2.2), but predicting pasture weed content was poor for all sites and seasons ($R^2 \leq 0.44$ and SD/RMSEP ≤ 1.2). The inaccurate predictions might be explained by the fact that the diversity found in the field and observed in the pasture spectral data was not taken into account in the pasture botanical separation.

The potential for using proximal sensing techniques to predict pasture nutrients *in situ* was confirmed, with the sensing of pasture N, P and K increased by the procedure of separating the data according to the season of the year. The full potential of the technology will only be realised if a substantial dataset representing all the variability found in the field is gathered. The importance of obtaining representative datasets that embrace all the biophysical factors (e.g. pasture type, canopy structure) likely to affect the relationship, when building prediction calibrations, was highlighted in this research by the variance in the predictions for the same nutrient using different datasets, and by the inconsistency in the number of common wavelengths when examining the wavelengths contributing to the relationship. The ability to use a single model to predict multiple nutrients, or indeed individual nutrients, will only come through a good understanding of the factors likely to influence any calibration function. It has been demonstrated in this research that reasonably accurate and precise pasture nutrient predictions ($R^2 > 0.74$ and SD/RMSEP ≥ 2.0) can be made from fresh *in situ* canopy measurements. This still falls short of the quality of the predictions reported for near infrared reflectance spectroscopy (NIRS) for dried, ground samples analysed under controlled laboratory conditions.

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List of Abbreviations

A	autumn
Alf.	Alfredton
ASD	Analytical Spectral Devices – ASD Inc.
Ati.	Atiamuri
Bal.	Ballantrae
CAPP	canopy pasture probe
c.f.	compare or consult
Col.	Colyton
Conc.	Concentration (%)
CV	coefficient of variation
DM	dry matter
FDR	first derivative reflectance
FIA	flow injection analysis
FR	full range
ICP-OES	inductively coupled plasma-optical emission spectroscopy
IR	infrared
LV	latent variable
Max.	maximum
Man.	Manawahe
Min.	minimum
MIR	mid infrared
NIR	near infrared
NIRS	near infrared reflectance spectroscopy
PCA	principal component analysis
PLSR	partial least squares regression
PTFE	polytetrafluoroethylene
RMSE	root mean square error
RMSECV	root mean square error of cross-validation
RMSEP	root mean square error of prediction

RPD	ratio prediction to deviation
Rua.	Ruakura
SD	standard deviation
Sp	spring
Su	summer
SWIR	short-wave infrared
Tok.	Tokoroa
Vis	visible
W	winter

CHAPTER 1:

General Introduction

1.1. Pastures

The grazing lands of the world are a major source of animal feed. Although the use of crops as livestock supplementation is growing, with the increasing emphasis on sustainable farming, pasture systems remain important, needing to be managed efficiently to sustain profitability (Riveros 1993). In New Zealand, legume-based pastures grazed *in situ* are the main source of feed for livestock (mainly the ruminants sheep, beef, dairy, deer and goats). Pasture is grazed all year round as a consequence of the warm and moist climate, which promotes pasture growth and enables ruminants to graze outdoors throughout the year (Guy 1993, Neeley and Parminter 1993). New Zealand‟s grasslands vary in composition, distribution and productivity according to changes in landform, climate, soil and nutrient and livestock management. Overall the soils are naturally acid with moderate to low chemical fertility, and current pasture production is based on genetically improved plants topdressed regularly with lime, and phosphate, sulfur and potassic fertilizers (Daly 1990). Pasture quality is a key determinant of animal productivity and farm profitability. Pasture quality is influenced by several management factors but principally vigorous clover and ryegrass growth driven by soil fertility and grazing management (White and Hodgson 1999). Managing high pasture quality through fertilizer inputs and pasture renovation incur the largest expenditure on most farms. Apart from a method for visual estimation of quality (Meat New Zealand 2009), farmers have no large scale objective management tool at hand to assist decisions on pasture quality management. Recently published literature (Dymond *et al.* 2006) suggest that near infrared reflectance spectroscopy, already used to assess the quality of dried forages in the laboratory (Roberts *et al.* 2004), could be applied in the field to supply farmers with “in the paddock” read out of pasture quality. Such information could be used directly to improve the efficiency of nutrient use via targeted fertilizer application or grazing management.

1.2. Remote sensing

There are several definitions of remote sensing, such as "remote sensing is the science or art of obtaining information about an object, area or phenomenon through the analysis of data acquired by a device that is not in contact with the object, area or phenomenon under investigation" (Lillesand *et al.* 2007); and "remote sensing consists of the interpretation of measurements of electromagnetic energy reflected from or emitted by a target from a vantage-point that is distant from the target" (Mather 1999). All definitions share the same central concept, that remote sensing is the gathering of information at a distance (Campbell 2007), and that distance defines the levels of remote sensing data collection, given that in order for a sensor to collect and record the electromagnetic energy reflected or emitted from a target on the Earth"s surface, it needs to be placed on a platform away from the surface being observed, and the platform can be based on the ground (laboratory or field), on an aircraft (airborne) or on a satellite (spaceborne) (Cracknell and Hayes 2007). To distinguish field remote sensing, from airborne or satellite, it has recently been referred to as proximal sensing.

1.3. Hyperspectral proximal sensing

In this thesis, hyperspectral proximal sensing (field remote sensing) is used to study the quality and quantity of pasture swards. Traditional multispectral remote sensing involves examination of features observed in several broad regions of the electromagnetic spectrum. Hyperspectral remote sensing examines many, very narrow contiguous spectral bands. Since hyperspectral data have more detail and greater accuracy it permits the observation of characteristics of vegetated surfaces (such as foliar chemistry) not feasible with multispectral data (Campbell 2007). Forty-two absorption features in visible and near-infrared wavebands have been related to particular foliar chemical concentrations (cellulose, chlorophyll *a* and *b*, lignin, nitrogen, oil, protein, starch, sugar and water), showing the relationship between plant chemical constituents and the absorption of electromagnetic radiation (Curran 1989). Remote sensing of plant biochemical constituents is complex, due to reflectance from vegetation being strongly influenced by the optical properties of plant materials. Different plant materials, like proteins, lignin, cellulose, etc, have similar absorptions, and a single absorption band can not be directly related to the chemical abundance of one single plant component (Kokaly and Clark 1999). The information on vegetation that can be extracted from remote sensing data depends on the capabilities of sensors, the knowledge about the interaction between radiation and vegetation canopy, and methodology used (Curran 1989).

Remote sensing techniques have proved to be useful for assessing the concentration of foliar biochemicals under controlled laboratory conditions. More investigation is required to assess their capabilities in the field (Mutanga *et al.* 2004). Hyperspectral remote sensing provides the potential for a significant jump in the quality of spectral data obtained about earth surface features (Lillesand *et al.* 2007), and recent studies show that the narrow bands may be crucial for providing additional information with significant improvements over broad bands in quantifying biophysical characteristics of vegetation (Thenkabail *et al.* 2000).

1.4. Objective

The objective of this study was to investigate the potential of hyperspectral proximal sensing to describe and quantify sward characteristics that are important in making critical decisions on the management of sheep and dairy pastures in New Zealand. The hypothesis is that sufficient sensitivity and consistency in the sensing of vegetated surfaces can be obtained *in situ* with hyperspectral proximal sensing, not possible with multispectral approaches, and such spectral data can be calibrated to pasture sward characteristics of economic importance. The resultant calibration should be robust and portable across a diversity of pasture swards. Specific objectives included: (a) develop a technique which enables the collection of *in situ* reflectance data from pasture canopies independent of ambient conditions; (b) evaluate the possibility of proximally sensing the botanical composition of grass-legume based pastures; and (c) explore the prediction of pasture nutrient concentration and mass of swards over contrasting soil fertility, landscapes (hill country site and lowland areas) and seasons of the year.

1.5. Outline

The thesis consists of 9 chapters, 7 of which are written and presented as standalone research papers. Two papers have been submitted to the International Journal of Remote Sensing: one has been accepted (Chapter 3) and the other is under revision (Chapter 2).

Chapter 1: *General Introduction.* Introduces the subject, the objective and structure of the thesis and briefly outlines each chapter.

Chapter 2: *A technique for acquiring in situ reflectance spectra from pastures independent of ambient conditions* (A paper from this study has been submitted to the International Journal of Remote Sensing – In review). New Zealand"s changeable weather and cloud cover represent a challenge for the timely collection of spectral data *in situ* under sunlight illumination. The Canopy Pasture Probe (CAPP) was developed to overcome this problem, to enable spectral data to be collected independently of ambient conditions. The Chapter describes the CAPP; how spectral measurements are obtained using the CAPP attached to an ASD FieldSpec® Pro FR spectroradiometer; provides examples of pasture swards measured using the CAPP; and shows the differences between canopy pasture spectra collected *in situ* using the CAPP compared to measurements under natural sunlight.

Chapter 3: *Large, durable and low-cost reflectance standard for field remote sensing applications* (A paper from this study has been published in the International Journal of Remote Sensing). The development of the CAPP required a suitable large reflectance standard. To purchase a traditional reflectance standard panel (such as Spectralon®) large enough to be used with the CAPP (greater than 20 cm diameter) would be expensive. This Chapter describes the search for a large, durable and low cost reflectance standard. A variety of materials (ceramic tiles, barium sulphate powders, white paint and a Kodak card) are evaluated, and a matt white ceramic tile was selected.

Chapter 4: *Influence of surface moisture on field hyperspectral data from pasture canopies*. The CAPP has been proven very efficient in controlling the illumination, and as a wind barrier to keep the target still during spectral measurements. Unfortunately not all atmospheric influences can be controlled under field conditions, and although the sunlight and wind are not a concern when using the CAPP, the target surface moisture can be. This Chapter examines the effect of surface moisture upon *in situ* hyperspectral data collected from pasture canopies.

Chapter 5: *Proximal sensing the botanical composition of New Zealand dairy and sheep pastures*. For pastoral farming systems, information on the botanical composition of swards is important in pasture management and in determining both the quantity and quality of forage. In this Chapter, the potential of using proximal sensing to obtain information on the botanical composition (grass, legume and weed contents) of New Zealand dairy and sheep pastures is evaluated.

Chapter 6: *Seasonal predictions of in situ pasture macro-nutrients in New Zealand pastoral systems using hyperspectral data*. In temperate regions of humid climatic regimes, the deficiency of one or more essential mineral nutrients is the most common limitation to optimum pasture growth. The ability to predict pasture nutrient content at critical times of the year is essential. This Chapter investigates the ability of proximal sensing to predict pasture macro-nutrients (nitrogen, phosphorus and potassium) in each of the four seasons of the year.

Chapter 7: *Estimation of nutrient concentration and mass of pastures grown under different soil phosphorus status and varying nitrogen fertiliser regimes using field spectroscopy*. The ability to predict pasture nutrient content *in situ* may vary for swards of different characteristics. A change in nutrient unit, such as the conversion of mineral concentration (percent of dry matter) into mass (kg ha^{-1}), might be an option to improve the proximal estimation of nutrients in forages. This Chapter explores the prediction (from reflectance factor spectra recorded *in situ*) of nutrient (nitrogen, phosphorus, potassium, calcium, magnesium, sodium and sulphur) concentration and mass by lowland dairy pastures swards with changing soil Olsen P status and N fertiliser inputs.

Chapter 8: *Prediction of the nutrient concentration and mass of hill pasture using proximal sensed hyperspectral reflectance*. Low soil fertility is a major factor limiting pasture production on hill country. Compared to lowland areas, hill pasture plant communities are much more complex, due to the high variability within very short distances. Slope and aspect influences livestock grazing, camping behaviour, soil fertility and the quality of the pasture. For appropriate management of hill pastures data from within paddocks are required to cover the diversity found in the field. This Chapter evaluates the prediction of mineral nutrient concentrations and masses (nitrogen, phosphorus and potassium) in hill country pasture swards on slopes > 20° and on north, east and south-facing aspects under sheep grazing, using hyperspectral proximal sensing.

Chapter 9: Summarizes the findings of this project and recommends future study directions.

CHAPTER 2:

A technique for acquiring *in situ* reflectance spectra from pastures independent of ambient conditions

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A paper from this study has been submitted to the International Journal of Remote Sensing: Sanches, I. D., Tuohy, M. P. and Hedley, M. J. (XXXX). A technique for acquiring *in situ* reflectance spectra from pastures independent of ambient illumination conditions. *International Journal of Remote Sensing*. **XX**, XXXX-XXXX. Submitted on May 2008. Revised on August 2009. Currently with referees.

Abstract

For the collection of pasture reflectance spectra in field conditions, the CAPP (canopy pasture probe) was developed. It consists of an inverted black plastic bin with a 50 Watt tungsten-quartz-halogen light source mounted on the top and a grip to insert the fibre optic input of an ASD FieldSpec® Pro FR. The independent light source allows acquisition of consistent reflectance spectra in variable or poor natural light conditions. Spectral measurements can be taken in different seasons of the year under identical conditions of illumination and view angle, allowing the acquisition of reproducible measurements. The CAPP has been successfully used to collect reflectance factor data from New Zealand pastures, a task that can be very difficult when relying on natural illumination because rapidly changing cloud cover and weather are characteristic conditions in this country.

Keywords: field spectroscopy; artificial illumination; field probe; pasture canopy

2.1. Introduction

Reflectance from vegetation surfaces is a complex phenomenon. There are several factors that influence vegetation spectral measurements, such as target reflectance properties (Knipling 1970), atmospheric conditions (Kriebel 1976), solar zenith angle (Kimes 1980), sensor characteristics, clouds and winds (Curtiss and Goetz 1994). Under natural conditions, cloud-free sky, minimal atmospheric turbidity and high sun elevations are required to acquire accurate spectral data (Williams and Wood Jr. 1987).

In the field, a practical and frequently utilized method to record reflectance measurements is to represent reflectance as a reflectance factor; this quantity is defined as the ratio of the radiant flux reflected by a surface to that reflected into the same reflected-beam geometry by an ideal, perfectly diffuse, standard surface irradiated under the same conditions (Nicodemus *et al.* 1977). However if the illumination conditions vary between the moment the reference and target materials are measured the resultant spectra will present errors (Curtiss and Goetz 1994).

New Zealand lies between latitudes 34° 04‟ S and 47° 02‟ S, and changeable weather is a common characteristic of mid-latitude areas, especially in maritime situations. Because of its oceanic position in the westerly wind belt, the atmosphere over this country is rarely still (Garnier 1958). Both the frontal systems across the ocean and the rugged landscape have a major influence on the New Zealand weather; over land and at the coast, clouds are formed through mechanical turbulence, convection and orographic ascent (Beatson 1985). An analysis of 30 localities in this country found that cloud frequency ranged from 56 to 92%. This estimation was based on daily NOAA7 satellite images over 347 days (not considering winter months), from November 1982 to April 1984 (Wardle 1986). Accordingly, the changeable weather and cloud cover present a great challenge for the successful use of passive optical proximal sensing technology in New Zealand.

One option to enable more control over illumination and to acquire measurements during non-optimal conditions is to use an artificial light source. Artificial illumination has been traditionally used in laboratory spectroscopy, but the collection and transportation of the samples to a laboratory can be time-consuming, undesirable or even impossible considering the destructive characteristic of the method.

To avoid the time-varying nature of the irradiance and to minimize the time spent between the collection of samples in the field and the measurement of spectral reflectance in the laboratory, Williams and Wood Jr. (1987) developed the Transportable Hemispherical Illumination System (THIS), a stable source of hemispherical illumination which could be transported to the area where the study would be conducted. The idea was to bring the laboratory to the samples, instead of the samples to the laboratory. More recently a range of contact devices has been developed by ASD Inc. for the measurement of small targets (e.g. leaves), and although the size of the objects that can be measured is limited, the integral light source of these devices permits measurements to be made in the field independently of the natural illumination, and moreover, the measurements are highly reproducible (Milton *et al.* 2007).

In our case the objective was to use a portable spectroradiometer (ASD FieldSpec® Pro FR - ASD Inc., Boulder, CO) to measure reflectance of pasture canopies in the field, using a non-destructive approach, in different seasons of the year, even under non-optimal conditions (e.g. cloud cover, wind). Since no existing device was suitable for our needs, the CAPP (canopy pasture probe) was developed. The CAPP is described in this paper, and it has been successfully used to collect reflectance factor data from dairy and sheep-grazed pastures on flat and steep topography, respectively. These data were collected to assess the feasibility of predicting pasture mineral nutrients using proximal sensing techniques.

Although the idea of taking the laboratory (controlled conditions) to the field is not new, to the authors‟ knowledge there is no other hand-held device currently being used which ensures a controlled environment for the use of spectroradiometers to measure pasture canopies *in situ* without destroying the target.

In the remaining text the term Spectro-CAPP was adopted to refer to the combined use of an ASD FieldSpec® Pro FR spectroradiometer attached to the canopy pasture probe.

2.2. Material and methods

2.2.1. CAPP (canopy pasture probe)

The CAPP consists of an inverted rounded black plastic bin, painted inside with a flat black paint; it is 45 cm high and has a diameter of 26 cm (Figure 2.1). Mounted in the top is a 50 Watt ASD Pro-lamp with a tungsten-quartz-halogen bulb. The light was placed in the top centre of the instrument at zenith, and it is powered by 12 volt battery. To insert the spectroradiometer fibre optic, a grip was initially fixed on the top of the probe beside the lamp, i.e. 45 cm from the target and pointing at approximately 11 degrees; later another grip was fixed on the side of the CAPP, at 40 cm height and 18 degrees from zenith. The field-of-view (FOV) of the spectroradiometer used is 25 degrees and the potential area viewed by the instrument (area measured) is very close to a circle with a diameter of approximately 20 cm. For the reflectance standard a square, matt white ceramic tile, measuring 295x295 mm was used (Sanches *et al*. 2009, Chapter 3).

Figure 2.1. (a) CAPP with the spectroradiometer ASD FieldSpec® Pro FR; (b) ASD light source mounted on the top and fibre optic cable grips on the top and side of the CAPP; (c) acquiring reflectance spectra from sheep-grazed pasture.

2.2.1.1. The frame

The CAPP was made of a resistant plastic suitable for field work conditions. Although the plastic used was already in black colour, the interior of the bin was painted with a flat black paint to minimize any reflectance from this surface. The paint used (Dulux Quick Dry™ Sparykote™) is spectrally non selective for the range analysed (350-2500 nm) and has very low reflectance (around 0.06) (the paint"s reflectance was measured using the ASD FieldSpec® Pro FR attached to an ASD plant probe and a spectralon® panel as reflectance standard). The CAPP‟s frame is light enough to be easily carried along with the backpack-mounted spectroradiometer; and most importantly, it blocks any interference from the outside environment during the measurements, such as secondary sources of illumination and wind.

2.2.1.2. Fiber optic input position

There are two types of reflectance from Earth-surface materials, specular and diffuse (Lambertian). For specular, the angle of incidence and reflection of the energy are equal and no scattering occurs at the surface. For diffuse reflectance, the radiance incident upon the surface is backscattered in all upward directions (Mather 1999). The diffuse component of reflectance from plant leaves comes mainly from inside the leaf, whereas the specular component arises primarily from the leaf surface (Grant 1987, Brakke 1994). The spectral variation of the diffuse reflection depends on leaf biochemistry and so can be used to predict the amount of leaf constituents (Bousquet *et al.* 2005). In most natural materials, like in vegetation targets, the diffuse reflectance is more abundant and causes fewer measurement problems (Hatchell 1999). Nevertheless, because the specular reflection depends on surface biophysical properties it can be useful for studies of refractive index and roughness of the epidermis/cuticle layer (Bousquet *et al.* 2005). Since the CAPP was developed to assist studies for the prediction of pasture biochemistry, our interest was to measure the diffuse reflectance and avoid the specular reflectance.

One way to avoid measuring specular reflectance is to set the spectroradiometer sensor input far away from the specular plane, for example by placing the sensor input at a right angle to the illumination source (Hatchell 1999). Since this set up of right angle was not compatible with the physical structure of the CAPP, two other set ups were tested. The choice for the set ups was guided by the objective to measure a circular area of at least 15 cm diameter (considering the FOV of the spectroradiometer of 25 degrees), with a probe that was practical for field work. Two different mounting positions for the FieldSpec® Pro FR fibre optic grip on the CAPP were tested. For position 1, the grip was fixed on the top of the probe (45 cm height) at 11 degrees to the light source (Figures 2.2a and 2.3a). For position 2, the cable grip was moved from the top to the side of the probe; it was placed at 40 cm height and 18 degrees from zenith (Figures 2.2b and 2.3b). The intention was not to have a probe with two different configurations, but to test if at least one of the probes would suit our needs.

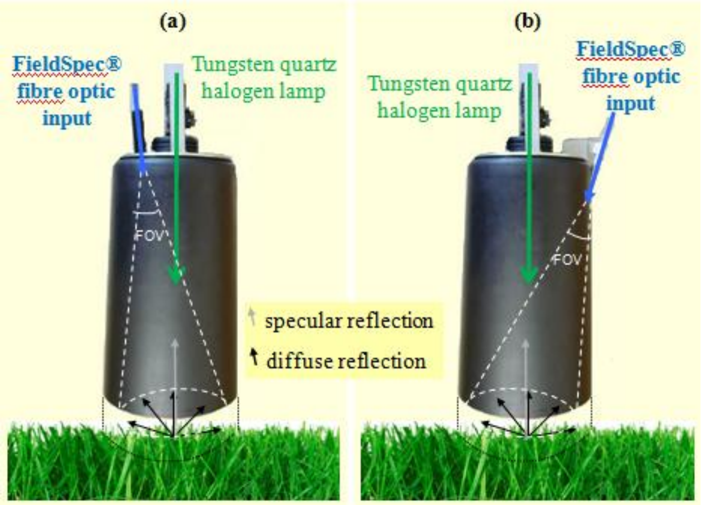

Figure 2.2. Diagram of pasture specular and diffuse reflection being captured by the CAPP with the ASD FieldSpec® Pro FR fibre optic input placed: a) on the top of the CAPP, b) on the side of the CAPP.

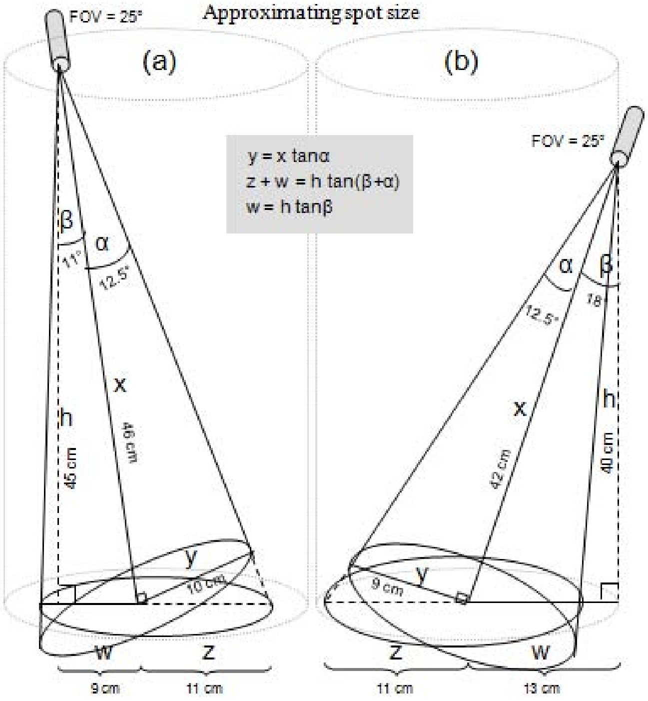

Figure 2.3. Calculating area measured within the FOV of the sensor for measurements acquired by the CAPP with the ASD FieldSpec® Pro FR fibre optic input placed: a) on the top of the CAPP, b) on the side of the CAPP.

The area measured by the Spectro-CAPP, as demonstrated in Figure 2.3, is slightly different for each set-up, but the potential ground area viewed by both set-ups corresponds closely to a circle with a diameter of approximately 20 cm. However, for both set ups the actual area measured will depend on the pasture sward height (PSH), since the distance between sensor input and target will decrease with any increase of the pasture height. The change in measured area by the Spectro-CAPP (top and side-grips) for a pasture sward with different heights is illustrated in Figure 2.4. One factor to be considered is that comparing two pasture plots of equal size, one with a sward higher than the other, the plot with higher sward might need more spectral measurements to be taken (measurements posterior averaged) in order to cover the same area (c.f. fig 2.4 PSH = 15 cm and PSH = 5 cm); especially for the side-grip set-up, where the area measured is slightly smaller than with the top-grip set-up.

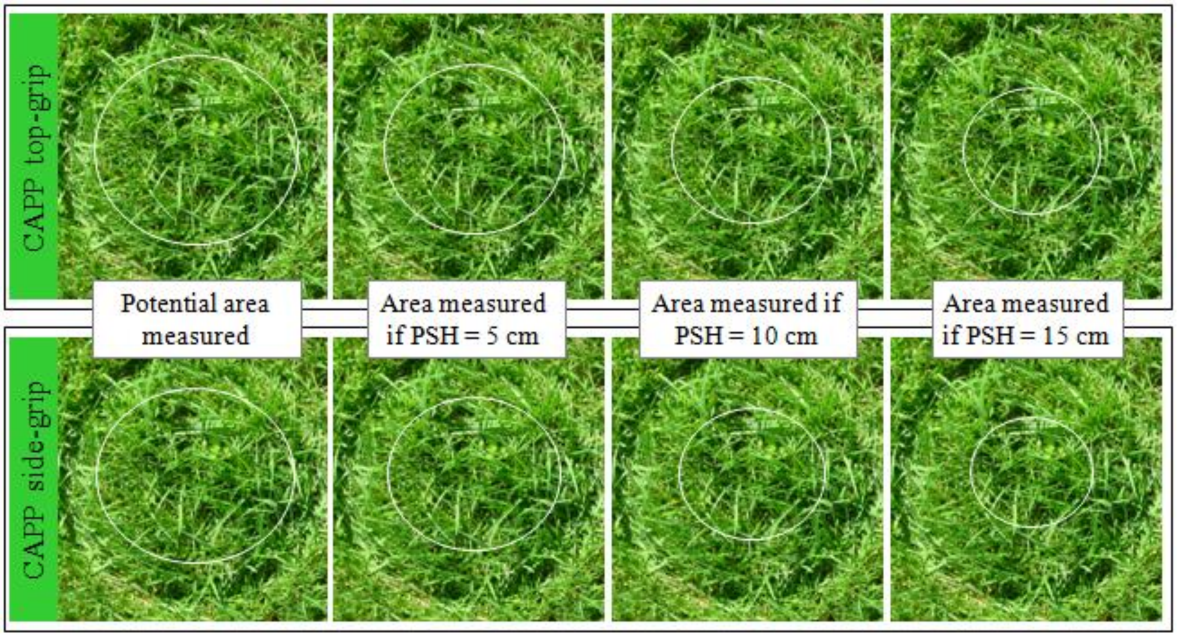

Figure 2.4. The area inside the white circles illustrates the approximate area measured by the Specto-CAPP using the fibre optic input placed on the top of the probe and on the side of the probe, for different pasture sward heights (PSH).

2.2.2. Light source

There are several kinds of radiant energy generators that can be used as artificial illumination sources, such as filament and gaseous emitters excited by electrical power, burning gaseous and solid emitters, or heated gaseous and solid emitters. The most appropriate option to obtain a smooth energy output when measuring spectral data between 350 and 2500 nm is a bulb with tungsten filament emitter, a non absorbing gas (halogen) and a non absorbing envelope (quartz) (Hatchell 1999).

The lamp mounted on the top of the CAPP was an ASD 50 Watt tungsten-quartz-halogen Pro-lamp (ASD Inc., Boulder, CO), which was set at maximum intensity in order to avoid noise problems. The stability of the light source was assessed. The spectral radiance of a matt white ceramic tile was measured at 1-minute intervals over 30 minutes using the Spectro-CAPP plugged in to mains power (as opposed to running on batteries).

Because the lamp is fixed, the distance between the light source and the target will vary with PSH. Consequently PSH will have an effect on the irradiance (amount of flux per unit area of a defined surface) on the target, the higher the PSH the higher will be the irradiance on the target. The spectral measurement of our interest is the reflectance factor, a ratio which is independent of the irradiance. So as long as the irradiance on the reflectance standard is the same as the irradiance on the target, the measurements will be coherent despite of PSH. This subject is further discussed in the text.

2.2.3. Battery

The CAPP‟s light source is powered by a 12 volt rechargeable battery. Since the overall goal for illumination is to keep it consistent, the battery stability was tested. The ideal would be to scan the reflectance standard just before each target sample is scanned to account for any variation in battery power, but because this is not always feasible when working in field conditions, it is important to know the change in radiance one can expect when dealing with a battery powered light source. The influence of variation in battery power was evaluated by using Spectro-CAPP to measure the spectral radiance of a matt white ceramic tile, at 1-minute intervals over 60 minutes, using three different fully-charged 12 volt batteries.

2.2.4. ASD FieldSpec Pro FR

The ASD FieldSpec® Pro FR is a spectroradiometer which covers the spectral range between 350-2500 nm, with spectral resolution of 3 nm for the region 350-1000 nm and 10 nm for the region 1000-2500 nm. The spectral resolution is the measure of the narrowest spectral feature that can be resolved by the instrument, and is different and independent from the spectral sampling interval (spacing between sample points in the spectrum). The sampling interval for the ASD FieldSpec® Pro FR is 1.4 nm for the region 350-1000 nm and 2 nm for the region 1000-2500 nm, with measurements automatically interpolated and reported in 1 nm intervals (ASD 2000).

2.2.5. Reflectance standard

Spectralon® (Labsphere, Inc.) is one of the most commonly used reflectance standards. It is made of a sintered PTFE material with almost perfect Lambertian reflectance over 250-2500 nm (Springsteen 1999). However, large panels (greater than 20 cm diameter) suitable for use with the CAPP are very expensive. Thus, a white matt ceramic tile was chosen as reflectance standard for the Spectro-CAPP (Sanches *et al*. 2009, Chapter 3). The selected tile has around 80% reflectance, presents good uniformity over the spectral range between 400 and 2500 nm and results in vegetation spectra with very similar shape to the spectra acquired using spectralon® as reference.

2.2.6. Acquisition and pre-processing of reflectance factor spectra acquired using the Spectro-CAPP

As mentioned before, the reflectance factor of a target is the ratio of the spectral response of the target to the spectral response of a reference sample under the same conditions of observation and illumination. Therefore the reference spectrum should be acquired before the target measurements begin, and should be repeated every time the illumination conditions change.

The FieldSpec® Pro FR spectroradiometer samples spectra continuously but reports a time averaged spectrum. To acquire the spectral reflectance of a target, the target must be kept in the FOV of the fibre optic cable until the reported time-averaged spectrum stabilises. The practice of time-averaging many spectra is adopted to reduce measurement error when collecting spectral data. The FieldSpec® Pro RS^3 software is set up to do this averaging. For our measurements one acquired spectrum is the average of 25 measurements for target, dark current and white reference spectra.

The measurement protocol for acquiring reflectance factor spectra using the Spectro-CAPP is as follows. Warm up the FieldSpec® Pro FR for at least 90 minutes. In the field turn on the CAPP‟s light (which is attached to a battery) and let it warm up for 15 minutes just before beginning the spectral measurements. Attach the FieldSpec® Pro FR‟s fiber optic into the CAPP, place the white reference sample (matt white ceramic tile) under the CAPP and proceed with the standard procedures to take relative reflectance (reflectance factor) measurements using the FieldSpec® Pro FR (optimization, collection of dark current and white reference scan). Once the white reference has been collected, place the CAPP over the target (pasture sward) and collect the target spectrum. Move the CAPP to the next target and acquire new spectrum. To measure plots with dimensions greater than the spot measured within the FOV of the Spectro-CAPP (Figure 2.3), several spectra are acquired per plot and posterior averaged. When collecting a target‟s spectra there is a need to periodically check the white reference scan (by placing the CAPP over the matt white ceramic tile), and collecting a new white reference scan whenever necessary (e.g. when deviations such as steps or slopes are observed in the 100% white reference line).

In the protocol described above, since the white reference scan is acquired by placing the ceramic tile under the CAPP (0 cm height), it is assumed pasture canopy height is short and constant. The ideal would be to have a mechanism to slide the reflectance standard panel up inside the CAPP according to the PSH. Thus the irradiance of both reflectance standard and target would be the same. Because the CAPP did not have such mechanism, and to manually change the tile height during measurements in the field would be unpractical, the reflectance factors collected using the Spectro-CAPP need to be posterior compensated for PSH. The radiance of the ceramic tile (which was previously cut to a size that could fit inside the CAPP) at different heights inside the CAPP (every centimetre between 0 to 15 cm height) was acquired (Figure 2.6). With these data - the radiance of the ceramic tile for specific heights and also measurements of PSH, the pasture reflectance factor measured with the Spectro-CAPP can be corrected. The correction is done by multiplying the pasture reflectance factor by the tile‟s radiance at 0 cm height and dividing by the tile‟s radiance at the specific PSH, for each wavelength. Another option would be to record in the field the radiance of the target instead of the reflectance factor, and posterior divide it by the tile‟s radiance at the specific PSH. However the advantage of recording the reflectance factor is that quality control of the spectra can be done visually while acquiring the data and some errors can be eliminated before the data are saved.

Following the methodology described above, the spectral reflectance factors from ten pasture plots were measured using the Spectro-CAPP. Each spectrum was the average of 10 spectra acquired within each plot. Data were obtained for the two fibre optic grip mounting positions. To remove the signal noise (mainly at the beginning and far end of the spectrum analysed, due to low signal-to-noise ratio - SNR) and step problems (steps discussed later in the paper) two data pre-processing procedures were applied to the spectra, a Savitzky-Golay smoothing filter (Savitzky and Golay 1964) with smoothing window size of 81 and polynomial order 4, using The Unscrambler® 9.7 software, and a de-step procedure (Daniel, P., Ticehurst, C., and Thulin, S., personal communication, 29 March 2007) using ENVI 4.3 plus IDL 6.3 (Figure 2.8). The de-step procedure assesses the actual differences at the steps between the three detectors in the spectrum, and then it takes the midpoint in each step as the new point for the corrected spectrum to pass through. Each of the three spectral segments is tilted up or down until they pass through the midpoints while the ends of the spectra (350 nm and 2500 nm) are held fixed. The authors later became aware of another de-step method called Splice Correction, available on the ViewSpec™ Pro software (ASD Inc.).

2.2.7. Comparison between pasture spectra acquired with the Spectro-CAPP and with the FieldSpec® under sunlight conditions

In addition to the measurements using the Spectro-CAPP, some pasture plots were also measured with the FieldSpec® in normal mode (under sunlight conditions) using as white references the matt white ceramic tile (295x295 mm) and a spectralon® disc (9 cm diameter). The fibre optic input when using the FieldSpec® under sunlight was fixed at nadir position at 45 cm height, so that the area measured (equal to a circle with a 20 cm diameter) would be comparable to the area measured when using the CAPP. For each plot four measurements were conducted: using 1) the FieldSpec® under sunlight and the spectralon® disc; 2) the FieldSpec® under sunlight and the ceramic tile; 3) the Spectro-CAPP top-grip and the ceramic tile; and 4) the Spectro-CAPP side-grip and the ceramic tile. For the inter-comparison of spectra collected using the two different reflectance standards used, the reflectance factor spectra obtained were converted to absolute reflectance (reflectance factor spectrum multiplied by the calibrated reflectance spectrum of the spectralon® disc or the ceramic tile).

2.3. Results and discussion

2.3.1. Fiber optic input position: grip fixed on the top of the CAPP versus grip fixed on the side of the CAPP

When the ASD FieldSpec® Pro FR fibre optic was inserted into a grip fixed on the top of the CAPP (45 cm height) at 11 degrees to the light source (Figures 2.2a and 2.3a), the resultant pasture spectra contained unwanted steps at 1000 and 1800 nm (Figure 2.5). Steps in field spectra are common in instruments that use multiple detectors and to discover the source of steps can be very difficult (Milton *et al.* 2007). The wavelengths 1000 and 1800 nm correspond to the splice points between the three detectors in the ASD FieldSpec® Pro FR instrument (VNIR, SWIR1 and SWIR2), and because the signal is low at splice points, the differences are most noticeable at those places (ASD 2000). The steps can occur when the spectroradiometer is not warmed up sufficiently before being used; but that was not the problem here as the machine was always warmed up for at least 90 minutes. When measuring targets with heterogeneous surfaces (vegetation canopies) the steps can also be related to the fact that each fibre in the fibre optic bundle of the ASD FieldSpec® Pro FR has its own field of view and the fibres are randomly distributed between each of the three detectors (MacArthur 2007, MacArthur *et al.* 2007).

When the cable grip was moved from the top to the side of the probe at 40 cm height and 18 degrees from zenith (Figures 2.2b and 2.3b), the area measured within the FOV was reduced slightly (Figure 2.3) and the step problems at 1000 nm and 1800 nm were minimized, as demonstrated in Figure 2.5. Overall there was an increase in reflectance factor values when using the sensor input on the side of the CAPP. It is important to emphasize that for both set ups, both target and white reference were measured with the same configuration, and the increase in reflectance factor is possibly related to the canopy structural effect.

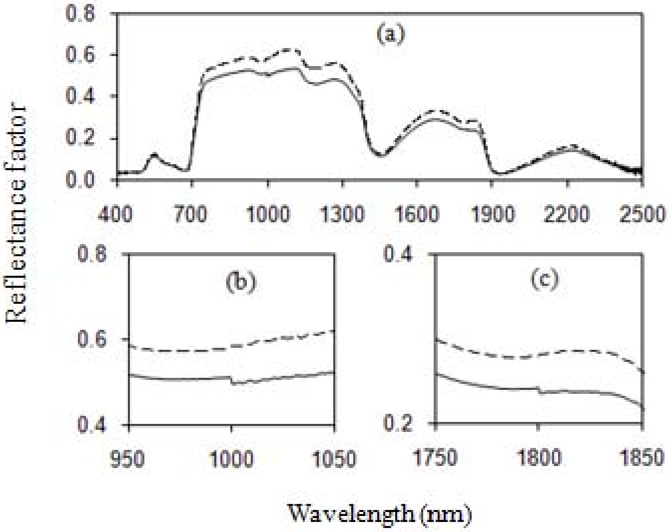

Figure 2.5. Spectra of a pasture measured with the Spectro-CAPP, using the fibre optic input placed on the top of the probe (continuous lines) and on the side of the probe (dashed lines), between a) 400 nm to 2500 nm, and enlarged for the spectral range between: b) 950 nm and 1050 nm; c) 1750 nm and 1850 nm.

2.3.2. Light source

The spectral radiance reflected from the reference sample (matt white ceramic tile) illuminated by the Pro-lamp mounted on the CAPP has the bell shape typical of a tungsten light source (Figure 2.6a). Overall the radiance values were low. The radiance results obtained here are similar to those of Williams and Wood Jr. (1987). They commented that low radiance values are to be expected when measuring reflected diffuse radiance, and not even sunlight has a flat and high radiant output across the entire optical portion of the electromagnetic spectrum.

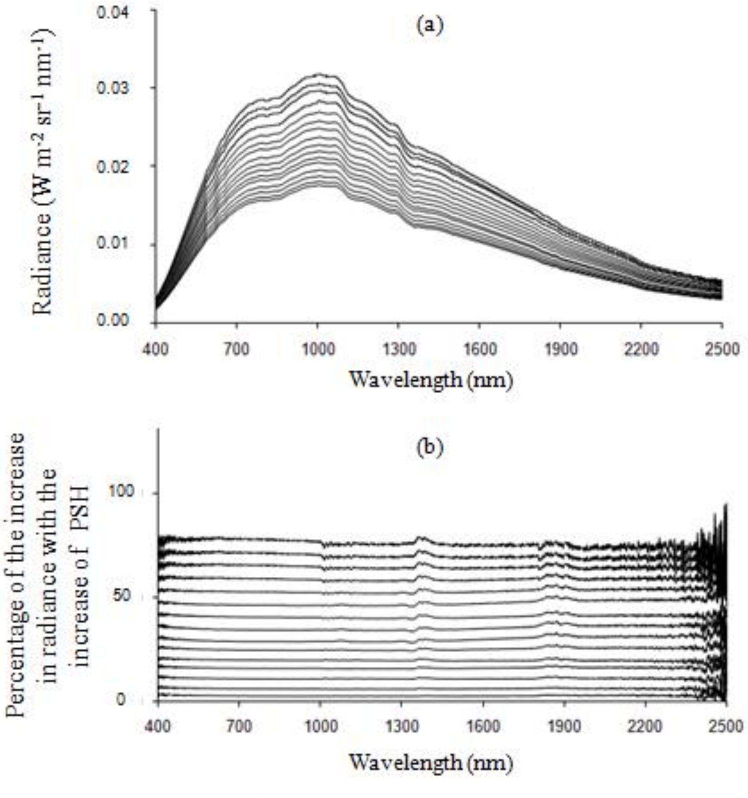

Figure 2.6. (a) Radiance (W m^{-2}sr^{-1}nm^{-1}) reflected from a reference sample (matt white ceramic tile) illuminated by the ASD 50 W Pro-lamp mounted on the CAPP. The 16 curves plotted correspond to the radiance of the reference sample when the reference panel was elevated to different heights (every centimetre between 0 to 15 cm) inside the CAPP: the curve with the lowest radiance corresponds to the reference sample placed on the ground (0 cm height), the curve with the highest radiance corresponds to the reference sample lifted 15 cm. (b) Percentage of the increase in radiance observed in the plot (a).

2.3.3. Light and battery stability

The stability of the illumination source is crucial when measuring reflectance factors of targets. The stability of an artificial light source even if plugged into the mains also depends on time, mainly when switching on the lamp; our light stability tests showed that a warm up period 10 and 15 minutes is necessary to stabilise the radiance of the lamp used in the CAPP, and after this period of time the lamp"s radiance was very stable.

Since the light source of the CAPP is powered by a 12 v battery it was important to evaluate the ability of the battery to provide constant power over time. Over a period of 60 minutes (the count of the 60 minutes started after 15 min of warm up time) the spectral radiance from the matt white ceramic tile changed most in the visible part (350-700 nm) of the spectrum analysed (350-2500 nm), and the change became smaller as the wavelength increased (Figure 2.7). The averaged results are presented for four wavelengths in Table 2.1. There was a change of -0.81% (at 2100 nm) to -2.93% (at 600 nm) in radiance values recorded over 30 minutes and a change of -5.51% (at 2100 nm) to -15.34% (at 600 nm) over 60 minutes. This equates to a decrease in radiance of 0.03% (at 2100 nm) to 0.10% (600 nm) per minute for the first 30 minutes and a decrease of 0.09% (at 2100 nm) to 0.26% (600 nm) after an hour of battery usage. The decrease in radiance for the first 30 minutes represents a reasonable stability, so the procedure for the CAPP is to scan the reference sample (reflectance standard) as frequently as possible, and keep the time between reference sample scans under 30 minutes.

Table 2.1. Averaged change in radiance (%) at 600 nm, 1100 nm, 1600 nm and 2100 nm, over 30 minutes and 60 minutes of battery usage.

	Change in radiance over		Change in radiance per minute for	
Wavelength	30 minutes	60 minutes	First 30 minutes	First 60 minutes
600 nm	-2.93%	-15.34%	-0.10%	-0.26%
1100 nm	-1.76%	-9.02%	-0.06%	-0.15%
1600 nm	-1.13%	-6.59%	-0.04%	-0.11%
2100 nm	-0.81%	-5.51%	-0.03%	-0.09%

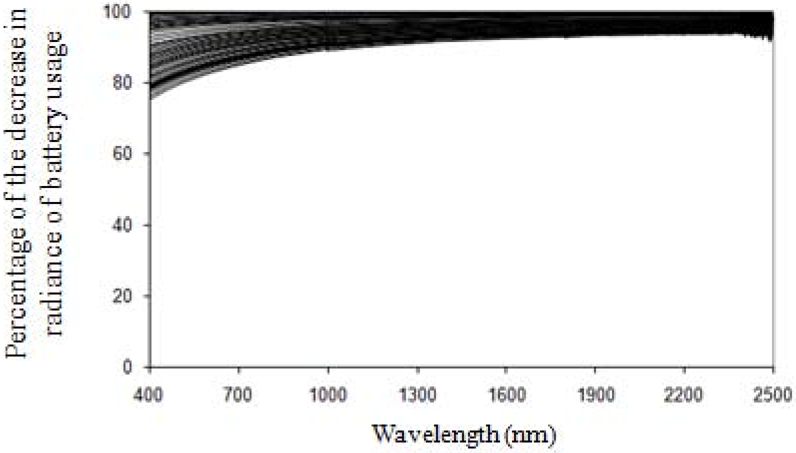

Figure 2.7. Average percentage decrease, over time, in radiance reflected from a reference sample (matt white ceramic tile) illuminated by the ASD 50 W Pro-lamp mounted on the CAPP powered by 12 v battery. The curves correspond to the decrease in radiance recorded per minute over a period of 60 minutes of battery usage (the count of the 60 minutes started after 15 min of warm up time).

2.3.4. Pasture reflectance factor acquired using the CAPP

The original spectra from pasture plots (before applying smoothing and de-step procedures) showed noise mainly at the longer wavelength end of the spectrum, and the steps previously mentioned, particularly the data collected with the top-grip (Figure 2.8a). After applying the smoothing filter the noise was minimized, and the steps were eliminated from spectra acquired with the side-grip. The steps, however, were much more significant at 1000 nm for the data acquired with the top-grip, and the smoothing procedure undesirably modified the original shape of the spectra around this wavelength (Figure 2.8b). The de-step procedure applied to the original data effectively eliminated the steps in all spectra (Figure 2.8c), and the de-stepping followed by the smoothing effectively eliminated the steps and minimized the noise (Figure 2.8d).

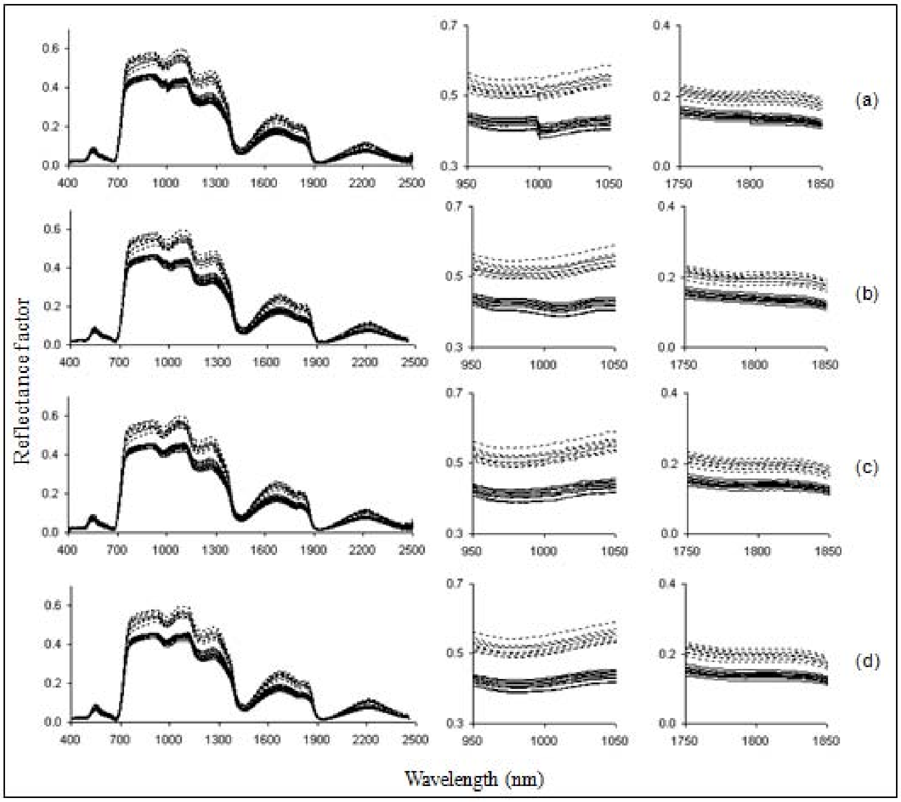

Figure 2.8. Spectra of pasture plots measured with the Spectro-CAPP using the fibre optic input placed on the top of the probe (continuous lines) and on the side of the probe (dashed lines), between 400-2500 nm and enlarged for the spectral ranges 950-1050 nm and 1750-1850 nm. a) Original data, b) smoothed data, c) de-stepped data and d) de-stepped and smoothed data.

Since the reference‟s radiance is acquired at 0 cm height (reference placed under the CAPP) independently of the height of the pasture sward being measured, and having in mind that in this study the pasture reflectance factor is the ratio between the pasture‟s radiance and the reference‟s radiance, the pasture reflectance factor spectra collected using the Spectro-CAPP need to be corrected according PSH. Otherwise, the pasture spectra will have higher magnitude than it should have, due to the increase in the ceramic tile‟s radiance with the increase of PSH (Figure 2.6). The degree of increase in the ceramic tile‟s radiance with the increase of PSH was the same all over the spectral range analysed (Figure 2.6b).

2.3.5. Comparison between pasture spectra acquired with the Spectro-CAPP and with the FieldSpec® under sunlight conditions

Some examples of the pasture plots measured using 1) the FieldSpec® under sunlight and the spectralon® disc; 2) the FieldSpec® under sunlight and the ceramic tile; 3) the Spectro-CAPP top-grip and the ceramic tile; and 4) the Spectro-CAPP side-grip and the ceramic tile are presented in Figure 2.9. The spectra illustrated in Figure 2.9 did not receive any smoothing procedure, but the spectra collected with the CAPP have been de-stepped. It is important to stress that the measurements presented in this study refer to the relative reflectance (reflectance factor) rather than the absolute reflectance. It is a relative reflectance because the calculation was based on a reference standard. When analysing spectra collected using different reflectance standards the absolute reflectance should be used. To allow comparisons between spectra collected using the spectralon® disc and the ceramic tile in Figure 2.9 the relative reflectance (not shown) was converted into absolute reflectance.

For the measurements using sunlight as the illumination source, the typical water bands noise at around 1400 nm and 1900 nm were observed. In each plot, the pasture spectra collected under solar illumination using the matt white ceramic tile and the spectralon® disc as white references were almost identical. The high degree of similarity of these absolute reflectance spectra was to be expected since the conditions of observation and illumination source were the same during the spectral measurements. The small dissimilarities observed between these spectra, mainly in plot A, are probably due to slight change in sunlight conditions during measurements. Just like the other examples illustrated in this paper, the spectra collected with the Spectro-CAPP top-grip had lower reflectance than the spectra acquired with the Spectro-CAPP side-grip. Overall, among the plots measured, all spectra acquired produced similar shapes with reflectance spectra varying in magnitude; it is important to remember here that the spectra being compared were collected under different view angles.

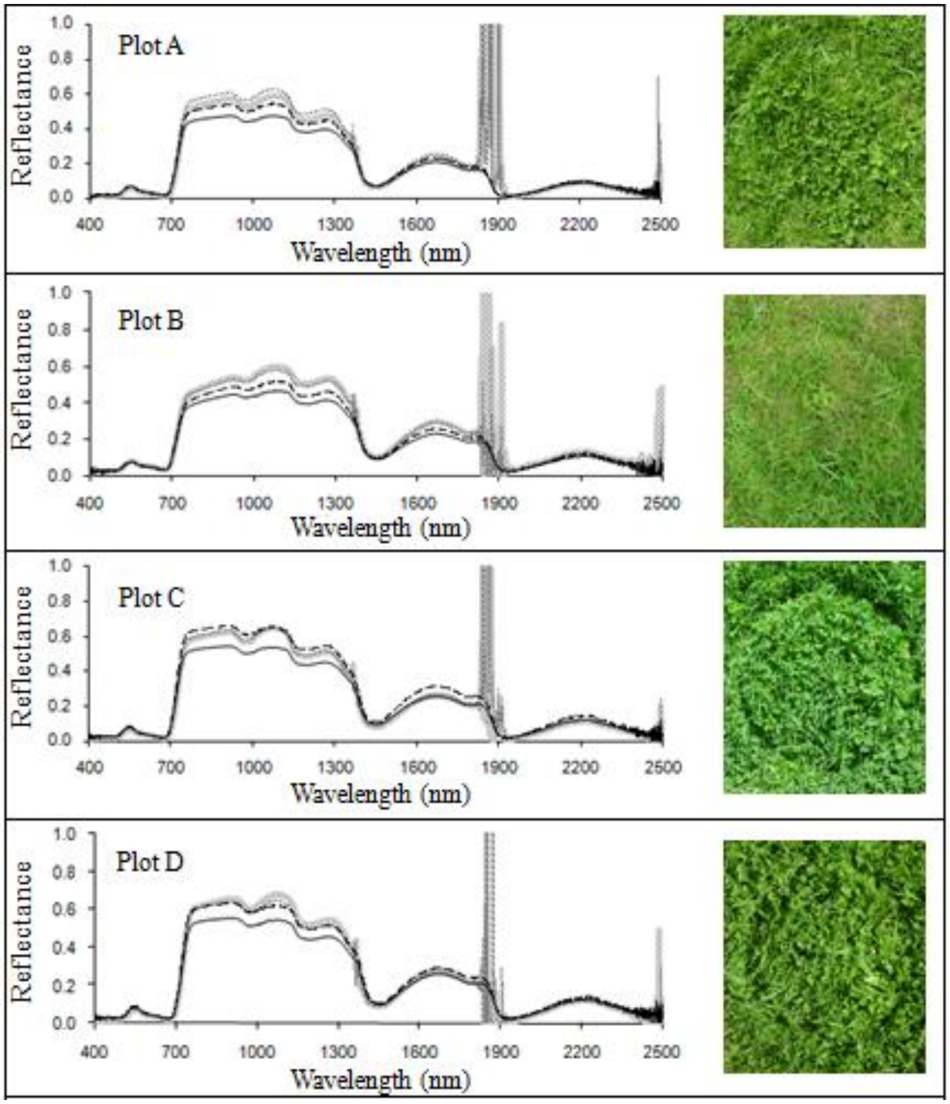

Figure 2.9. Reflectance spectra (relative reflectance converted into absolute reflectance) of different pasture plots (A, B, C and D) acquired in four distinct ways: 1) matt white ceramic tile as white reference and the Spectro-CAPP top-grip (black lines), 2) ceramic tile and the Spectro-CAPP side-grip (dashed black lines), 3) ceramic tile and the FieldSpec® under sunlight (dotted grey lines), and 4) spectralon® disc as white reference and the FieldSpec® under sunlight (thick grey lines).

2.3.6. Examples of pasture swards measured using the CAPP

To demonstrate the potential of the CAPP to measure spectra from a variety of pasture swards, some examples of pasture spectra acquired with the Spectro-CAPP using the fibre optic input placed in the top grip on the CAPP, are presented in Figure 2.10 (spectra have been pre-processed i.e. de-stepped and smoothed). Measurements were taken at two quite different sites in the North Island of New Zealand. Site one corresponds to a sheep pasture in steep hill country (low fertility) with browntop (*Agrostis capillaris)* and fescue (*Festuca spp.*) as the most common grass species. Site two corresponds to a dairy farm in a flat, lowland area (high fertility) with perennial ryegrass (*Lolium perenne*) as the dominant grass species. At each site, the same pasture plot was measured in different seasons of the year. In New Zealand the seasons have a great influence on pasture attributes, such as botanical composition, biomass, greenness, dead matter, etc. The changes in pasture characteristics were clearly reflected in the spectral measurements.

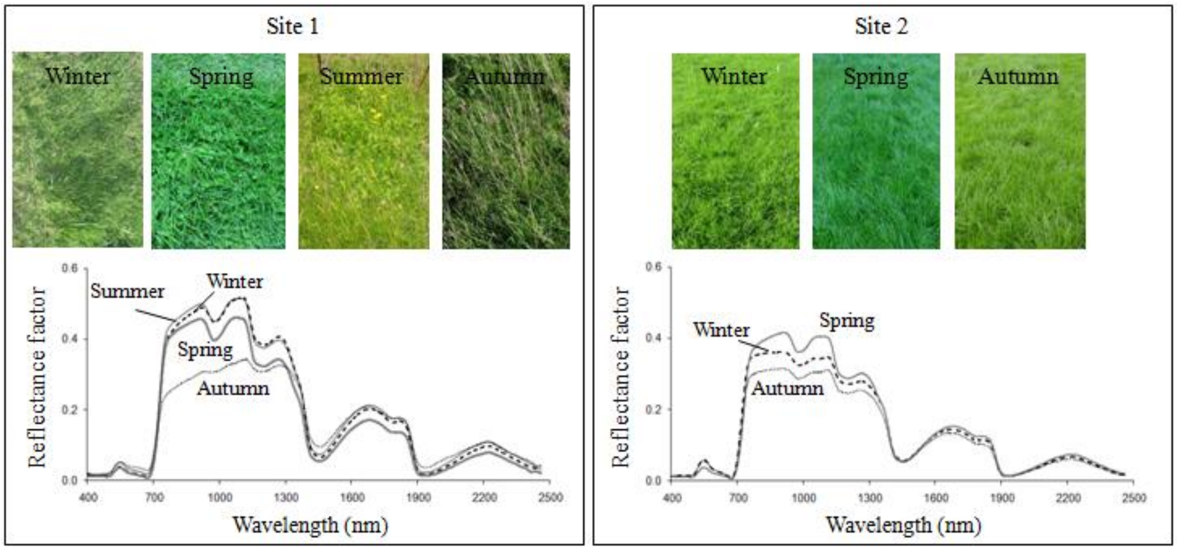

Figure 2.10. Examples of pre-processed (de-stepped and smoothed) reflectance factor spectra of pasture swards measured with the Spectro-CAPP using the fibre optic input placed on the top of the CAPP. Sites 1 and 2 correspond to a sheep pasture on a hill country site and a dairy pasture in a flat area, respectively. The pictures of each site show how the same pasture plot appears in different seasons of the year.

2.4. Conclusions

We have successfully developed a canopy pasture probe (CAPP) that can be used as an attachment to the fibre optic input of a portable field spectroradiometer. The CAPP has been used to acquire reflectance factor data from New Zealand pasture swards on flat and hilly farms. The advantages of using this device are that the independent light source allows the user: to acquire reflectance spectra from pasture in all seasons and variable weather conditions (e.g. cloudy sky, windy days) that are typical of temperate regions like New

Zealand; to measure pasture characteristics in the field without the need to cut samples and transport them to the laboratory; and to allow spectral measurements to be taken in different seasons of the year under identical conditions of illumination and view angle, a desirable trait when studying field plots over a period of time.

Like any other spectral data, the reflectance factor spectra measured using the Spectro-CAPP need to be pre-processed to remove, or at least reduce, undesirable features in the target spectra. After pre-processing (de-stepping and smoothing), both top-grip and side-grip data are good for further interpretation.

CHAPTER 3:

Large, durable and low-cost reflectance standard for field remote sensing applications

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A paper from this study has been published: Sanches, I. D., Tuohy, M. P., Hedley, M. J. and Bretherton, M. R. (2009). Large, durable and low-cost reflectance standard for field remote sensing applications. *International Journal of Remote Sensing*. **30**, 2309-2319.

Abstract

The development of the Canopy Pasture Probe (CAPP), for acquisition of *in situ* pasture canopy reflectance factors, required a suitable large reflectance standard. Spectralon® has been successfully used worldwide as a reflectance standard, but large panels (greater than 20 cm diameter) suitable for use with the CAPP are very expensive. In this context, a large, durable and low cost reflectance standard has been evaluated for use with an ASD FieldSpec® Pro FR spectroradiometer attached to the CAPP. In this study various ceramic tiles, barium sulphate powders, white paint and a Kodak card were tested. The material which best suited the requirements for a reflectance standard was the white ceramic tile sourced from Argentina. This tile produced around 80% total reflectance with reasonable uniformity over the spectral range analysed (400 – 2400 nm); and the vegetation spectra acquired using this tile as reference sample were very similar in shape to the vegetation spectra acquired using spectralon® as a white reference.

Keywords: reflectance standard; hyperspectral; ceramic tile; pasture canopy

3.1. Introduction

Physicists, chemists and biologists use laboratory spectroscopy to characterize the composition of materials based on the interaction of electromagnetic radiation with matter.

Imaging spectroscopy (also known as hyperspectral remote sensing) was developed following the same concept, however at a greater scale, using the solar electromagnetic spectrum (Green *et al.* 1998). In several remote sensing applications, imaging spectroscopy data need to be calibrated to surface reflectance, which is done from proximally sensed (laboratory and/or field) reflectance from known targets (Clark *et al.* 2002). In addition to calibration, field spectroscopy can also be used to assist the development of remote sensing techniques and feasibility testing (Curtiss and Goetz 1994).

In laboratory and field spectroscopy, the reflectance characteristics of the target are inferred by comparing the spectral response of the target to the spectral response of a reference sample (reflectance standard). In the field such measurements can be made under artificial or solar illumination by using a spectroradiometer, like the FieldSpec® Pro FR.

It is important to note that the real quantity acquired by the FieldSpec® Pro FR is the reflectance factor. The reflectance factor is the ratio of the radiant flux reflected by a target to that reflected into the same reflected-beam geometry by an ideal and diffuse standard surface, irradiated under the same conditions. This differs from true reflectance, the ratio of the reflected flux on a sample surface to the incident flux from the same surface (Schaepman-Strub *et al.* 2006). Only when the reflectance standard is a 100% reflecting panel does the reflectance factor approach true reflectance, however, it seems to be commonly accepted in the remote sensing community that measurements of reflectance factor taken using high reflecting reference materials, like 99% spectralon®, are normally called reflectance.

The reflectance factor can be converted to absolute reflectance in post-processing, by multiplying the reflectance factor spectrum by the actual calibrated reflectance spectrum of the reference standard (ASD 2000).

The ideal reflectance standard is a perfect reflecting diffuser, which is defined as a Lambertian diffuser with a reflectance factor equal to unity (Nadal and Barnes 1999). But, since there is no material with such characteristics, one that approximates to this concept is considered a good standard.

To be a reflectance standard a material should have the following properties: a reflectance factor close to unity for the operational wavelength range, be a diffuse reflector, highly opaque, non fluorescent, homogeneous with a smooth surface, spectrally non selective, durable, transportable, stable, cleanable, easy to handle (Nadal and Barnes 1999, Springsteen 1999) and weather-resistant (Jackson *et al*. 1992).

Some materials which have been used worldwide as reflectance standards are pressed and sintered polytetrafluorethylene (PTFE) resins, matt ceramic tiles and barium sulphates. They all have excellent reflection properties (Shitomi and Saito 2005).

Spectralon® (Labsphere, Inc.) is one of the most commonly used reflectance standards. It is made of a sintered PTFE material with almost perfectly Lambertian reflectance over 250-2500 nm (Springsteen 1999). The great disadvantage of spectralon® is its high cost, especially when a large panel is required.

Searching for an alternative for sintered PTFE (e.g. spectralon®), Knighton and Bugbee (2004) tested mixtures of barium sulphate with white latex paint. The idea of adding paint was to make the standard more durable, because the barium sulphate, although highly reflective, is easily rubbed off any surface. The paint mixed with barium sulphate did increase its durability, but it also decreased the reflectance.

Hanna *et al.* (1999) used a Kodak grey card as reflectance standard to estimate New Zealand pasture biomass using a three-channel radiometer (green, red and near-infrared). The grey side of the card turned out to be a better standard to use in the field than the white side of the card.

The objective of our research was to find a large (over 20 cm diameter), durable and low cost reflectance standard which could be used to obtain field pasture reflectance over the range of 400-2400 nm, using an ASD FieldSpec® Pro FR attached to the Canopy Pasture Probe (CAPP).

The CAPP (Chapter 2), Canopy Pasture Probe, consists of an inverted black plastic bin, with a light source mounted on the top (at zenith) and an input for the spectroradiometer fiber optic placed at 18 degrees from zenith (Figure 3.1). It was developed to allow the acquisition of weather-independent spectral measurements of pastures in the field (e.g. even on cloudy days) using a FieldSpec® Pro FR. Since the field-of-view (FOV) of the fibre optic cable input to the spectroradiometer used is 25 degrees, and the distance from the target is set to 40 cm, the area viewed by the instrument (spot measured) is elliptical with a major axis of 19.7 cm and a minor axis of 18.7 cm. Consequently a reflectance standard with dimensions greater than these was required.

3.2. Material and methods

3.2.1. Reflectance standards

The materials tested as reflectance standards used in this study and their costs (in New Zealand dollars) are reported below. The prices quoted represent the real amount spent to acquire the materials or the cost estimated at current prices (2006).

Spectralon® - Labsphere, Inc.:

- ***(1)*** 99% reflectance factor, 9 cm diameter

(To acquire a 99% reflectance factor spectralon® with dimensions of 250x250 mm - $2874)

Barium sulphate (BaSO4) - white powder:

- ***(2)*** May & Baker barium sulphate - $50/500g
- ***(3)*** PANREAC barium sulphate for X radiology - $198/5kg

White paint:

- ***(4)*** Dulux weathershield X10 low sheen acrylic - ($49/l)

Ceramic tiles - all white tiles except number 7 which is grey ($4-$6/tile):

- ***(5)*** Asia Tile (made in Indonesia)
- ***(6)*** Niro Whites
- ***(7)*** Cesi Perla Satin (made in Italy)
- ***(8)*** Tau Ceramica Benedresa (made in Spain)
- ***(9)*** Milenio Blanco Mate Wall T.T.
- ***(10)*** San Lorenzo Blanco Niveo (made in Argentina)
- ***(11)*** Nordico Snow

Card – Kodak Gray Card ($25):

- ***(12)*** the grey side of the card
- ***(13)*** the white side of the card

Both barium sulphate powders were manually pressed on two flat plastic dishes of 9 cm diameter. The May & Baker barium sulphate (2) was also manually pressed on a flat ceramic dish of 25 cm of diameter, and for that approximately 300 grams of barium sulphate were used. The white paint was painted on a piece of smooth-surfaced board; it was determined that the surface on which the paint was applied did not affect the spectral measurements of the paint.

3.2.2. Reflectance spectrometry

The evaluation of materials was conducted using the FieldSpec® Pro FR attached to: an ASD plant probe, and the CAPP (Figure 3.1).

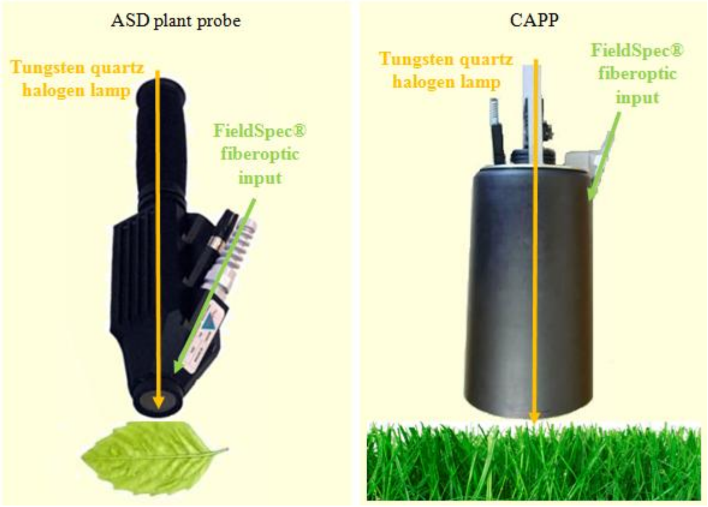

Figure 3.1. ASD plant-probe and CAPP (canopy pasture probe).

The reflectance spectra of all materials (barium sulphates, matt white paint, ceramic tiles and Kodak card) were compared to the spectralon®, which was considered the ideal reflectance standard because of its almost perfectly Lambertian reflectance characteristics over 400-2400 nm and high durability in field conditions. The largest spectralon® panel available was a 9 cm diameter disc, therefore the first tests were run using the plant probe. After those first comparisons, the materials considered good enough were tested using the CAPP.

3.2.2.1. FieldSpec® Pro FR attached to the ASD plant probe

The comparisons between spectralon® and the other materials mentioned above were achieved by analysing their reflectance (reflectance factor acquired using a calibrated Labsphere certified 99% reflectance standard (SRT-99-050)), the reflectance factor spectra of vegetation targets (fresh green leaves) obtained using the materials as reflectance standard, and their white reference scans. Each spectrum displayed is the average of 10 recorded spectra and was analysed using SpectraProc (Hueni and Tuohy 2006).

The white reference scans, which enable the adjustment of the instrument gain for optimal performance and determine the dark-offset used for automatic subtraction from reflectance calculations (ASD 2000), were acquired with the spectroradiometer viewing the incident illumination that was reflected off the materials tested as reflectance standard.

3.2.2.2. FieldSpec® Pro FR attached to the CAPP

For the materials not discarded in the previous phases, vegetation target reflectance factors were obtained using the FieldSpec® Pro FR with the CAPP and the test materials used as reflectance standard.

3.3. Results and discussion

3.3.1. Evaluation of a range of potential reflectance standards

The ceramic tiles and barium sulphates were chosen to be examined in this research because they have been used previously as reflectance reference samples. The grey side of the Kodak card, used for determining proper exposure in photography, videography and digital imaging, has been used as reference standard for inferring the reflectance from New Zealand pastures in the visible and near-infrared wavelengths (Hanna *et al.* 1999). A matt white paint was also tested following the idea of a pre-existing study which tested mixtures of barium sulphate and white latex paint (Knighton and Bugbee 2004).

With the exception of the spectralon®, all other materials investigated in this research could be used as large reflectance standards without great cost. The reflectance spectra of the tested materials acquired using the FieldSpec® Pro FR with the plant probe using a SRT-99-050 reflectance standard, (400-2400 nm) are shown in Figure 3.2. The grey card *(12)* and grey ceramic tile *(7)*, as expected, had the lowest reflectance factors compared to the other materials (all white coloured); the white paint *(4)* and the white card *(13)* had high reflectance values from 400-1400 nm, but at longer wavelengths their reflectance started to decrease (Figure 3.2b). All other materials had satisfactory high reflectance. Spectralon® *(1)* and barium sulphates *(2, 3)* had reflectance over 90% (except for the bariums at 1900 nm); the ceramic tiles Asia Tile *(5),* Niro Whites *(6),* San Lorenzo *(10)* and Nordico Snow (*11)* presented values between 80% and 90%; and the ceramic tiles Tau Ceramica (*8)* and Milenio Blanco (*9)* between 70% and 80% (Figure 3.2a).

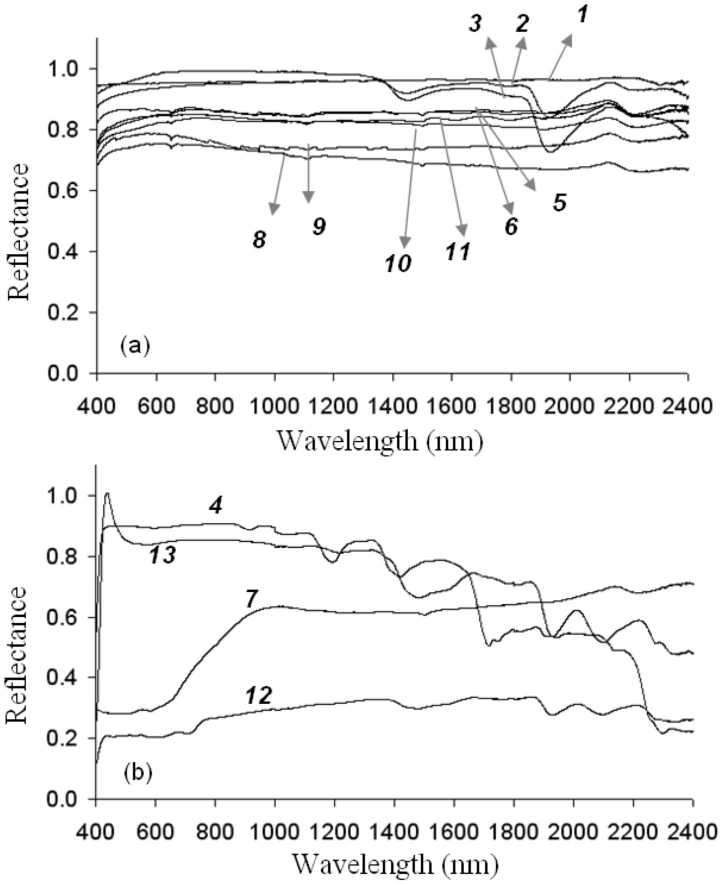

Figure 3.2. Reflectance of the materials inferred using an ASD FieldSpec® Pro FR with an ASD plant probe using a SRT-99-050 reflectance standard: a) spectralon® *(1)*, barium sulphates *(2* and *3)* and all white ceramic tiles *(5, 6, 8, 9, 10* and *11)*; b) white paint *(4)*, grey ceramic tile *(7)*, grey and white cards *(12, 13).*

One important property the standard should have is to be spectrally non-selective, that means its values for reflectance factors should be as uniform as possible in the operational wavelength range. The white paint *(4)*, the grey ceramic *(7)* and white card *(13)* were highly selective, and the grey card *(12)* was slightly selective (Figure 3.2b). The reflectance spectra of both barium sulphates *(2* and *3)* had troughs around 1450 and 1950 nm; whereas all white ceramic tiles *(5, 6, 8, 9, 10* and *11)* had relatively uniform reflectance spectra in the wavelength range analysed and the spectralon® *(1)* was the most uniform of all materials (Figure 3.2a).

Based on either spectral non-selectivity or generally low reflectivity in the wavelength range 400-2400 nm the grey ceramic tile *(7)*, white paint *(4)*, white card *(13)* and the grey card *(12)* were eliminated as possible reflectance standards for the purpose of calibrating the larger target area of the CAPP.

The remaining materials (spectralon® *(1)*, barium sulphates *(2, 3)*, white ceramic tiles Asia Tile *(5)*, Niro Whites *(6)*, Tau Ceramica *(8)*, Milenio Blanco *(9)*, San Lorenzo *(10)* and Nordico Snow *(11)*) were used as white references standards to record the spectra of a fresh green leaf. For the FieldSpec® Pro FR with the plant probe, generally the greatest variation in reflectance spectra produced using different reference materials was observed at the infrared wavelengths (Figure 3.3). The leaf reflectance factors acquired against the barium sulphates *(2* and *3)* were, in magnitude, the most similar to the spectralon® *(1)* result (Figure 3.3a), while the spectra referenced to the white ceramic tiles Asia Tile *(5)*, Niro Whites *(6)* (Figure 3.3b), Tau Ceramica *(8)*, Milenio Blanco *(9)*, San Lorenzo *(10)* and Nordico Snow *(11)* presented higher values. The difference in magnitude in the leaf spectra was predictable, because the spectralon® panel and barium sulphates are better reflectors than the ceramic tiles (Figure 3.2). This means that in the calculation of the leaf reflectance factor using those three materials as the standard, the denominator will have a greater value and the calculated reflectance factor will be less than when using the ceramic tiles.

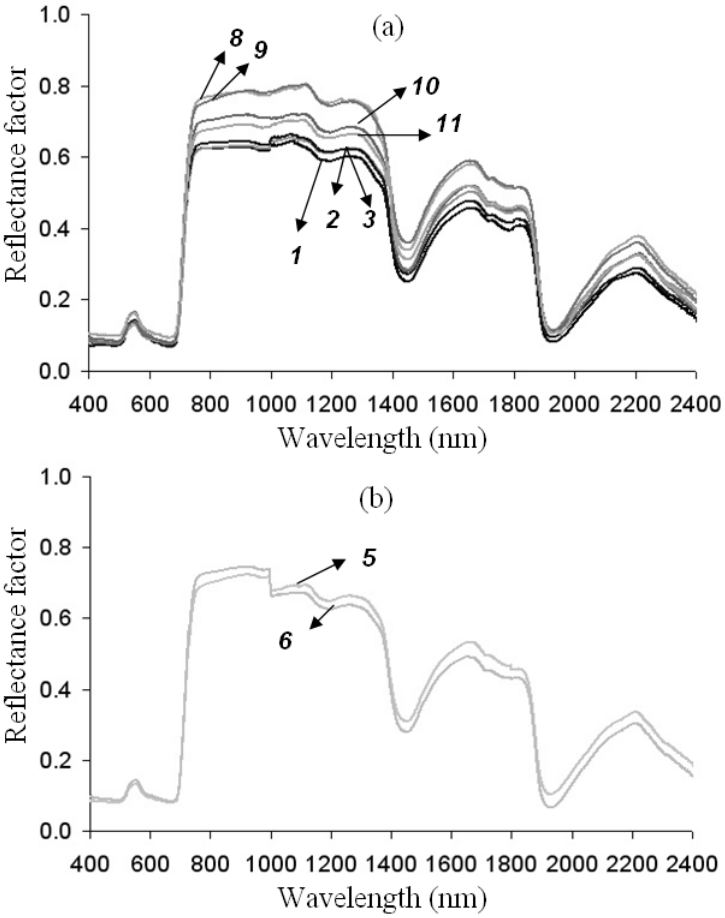

Figure 3.3. Spectra of a fresh green leaf obtained using an ASD FieldSpec® Pro FR with an ASD plant probe using as reflectance standard: a) spectralon® *(1)*, barium sulphates *(2, 3)*, white ceramic tiles Tau Ceramica *(8)*, Milenio Blanco *(9)*, San Lorenzo *(10)*, Nordico Snow *(11)*; b) white ceramic tiles Asia Tile *(5)* and Niro Whites *(6)*.

The white ceramic Asia Tile *(5)* had a marked „step“ at 1000 and 1800 nm and the Niro Whites *(6)* at 1000 nm (Figure 3.3b), and therefore they were discarded as reflectance standards. The spectralon® *(1)*, barium sulphates (*2* and *3*) and white ceramics tiles Tau Ceramica *(8)*, Milenio Blanco *(9)*, San Lorenzo *(10)* and Nordico Snow *(11)* showed no step feature (Figure 3.3a).

The features observed at 1000 and 1800 nm on the spectra of Figure 3.3b are known as steps at splice points. These wavelengths correspond to the splice points between the three detector spectrometers in the FieldSpec® Pro FR instrument (VNIR, SWIR1 and SWIR2). Because the signal is low at splice points, the differences are most noticeable at those places (ASD 2000). If the spectroradiometer is not warmed up sufficiently (at least 90 minutes) before being used, the step problem at 1000 and 1800 nm is very pronounced; this was not the problem here as the machine was properly warmed up. Factors causing the step problem are discussed later in the text.

Despite the differences in magnitude, the shapes of the leaf spectra acquired using spectralon® *(1)*, barium sulphates (*2* and *3*) and white ceramics tiles Tau Ceramica *(8)*, Milenio Blanco *(9)*, San Lorenzo *(10)* and Nordico Snow *(11)* (Figure 3.3a) were all visually very similar. Also their white reference scans were very alike (Figure 3.4) with noise occurring mainly near 2400 nm (low signal-to-noise ratio (SNR)) but even the spectralon® spectrum had similar noise.

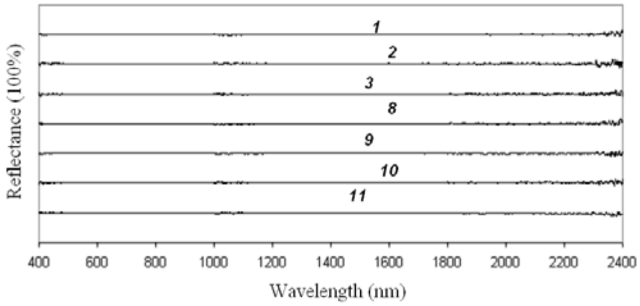

Figure 3.4. Offset by 10% of the white reference scans of spectralon® *(1)*, barium sulphates *(2*, *3)*, white ceramic tiles Tau Ceramica *(8)*, Milenio Blanco *(9)*, San Lorenzo *(10)* and Nordico Snow *(11)* taken using an ASD FieldSpec® Pro FR with an ASD plant probe.

The final comparison between spectralon® and the potentially suitable selected materials was done using the FieldSpec® Pro FR with the CAPP. The spectralon® panel *(1)* available (9cm diameter) could not cover the area viewed by the CAPP and was not used in this experiment. The barium sulphates for X-radiology *(3)* was also not used in this stage, because both barium sulphates analysed presented similar results, it was decided to make only one big plate using the May & Baker barium sulphate *(2)*.

Some differences between the spectra obtained using the plant probe and the CAPP were expected. Not only because the setup (illumination and viewing geometry) of both devices was slightly different but also because while the area viewed by the plant probe was filled by a single leaf, the CAPP was developed to look at (pasture) canopies.

The reflectance factors of a grass canopy obtained by the FieldSpec® Pro FR with the CAPP using as reflectance standards the *(2)* May & Baker barium sulphate, white ceramic tiles *(8)* Tau Ceramica, *(9)* Milenio Blanco, *(10)* San Lorenzo and *(11)* Nordico Snow are shown in Figure 3.5. The steps at splice points, that were observed for white ceramics Asia Tile *(5)* and Niro Whites *(6)* when acquiring the vegetation spectra using the plant probe (Figure 3.3b), were also a problem at 1000 and 1800 nm for the Tau Ceramica *(8)* (Figure 3.5) and at 1000 nm for the Nordico Snow *(11)* (Figure 3.5b) when using the CAPP. The May & Baker barium sulphate *(2)*, Milenio Blanco *(9)* and white ceramic San Lorenzo *(10)* did not have this problem.

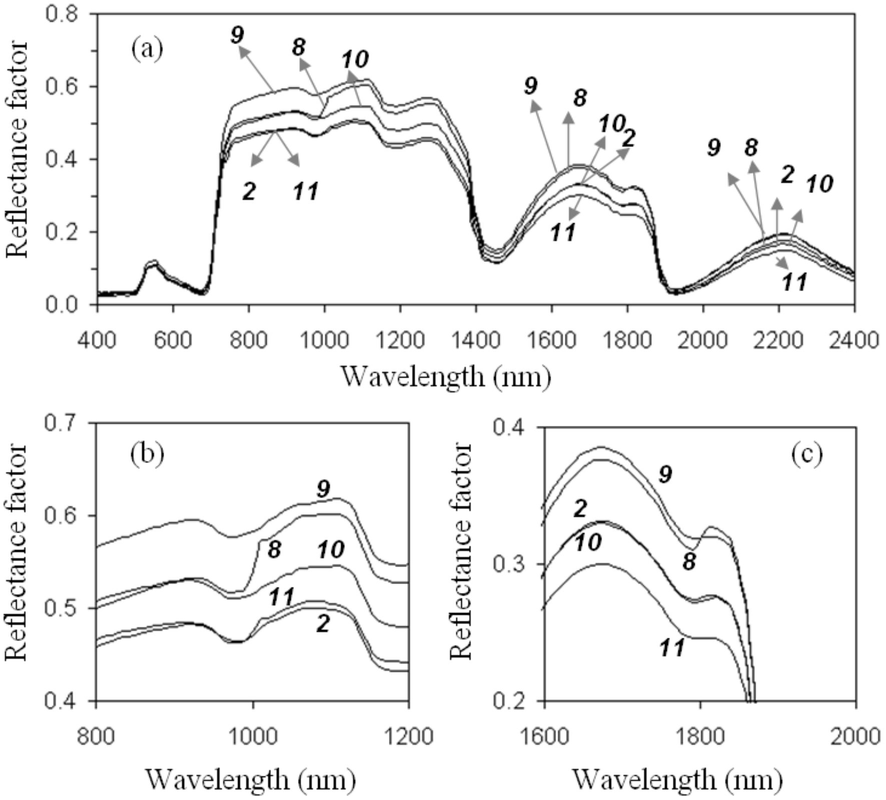

Figure 3.5. Spectra of a grass canopy acquired by an ASD FieldSpec® Pro FR with the CAPP, using the May & Baker barium sulphate *(2)*, white ceramic tiles Tau Ceramica *(8)*, Milenio Blanco *(9)*, San Lorenzo *(10)* and Nordico Snow *(11)* as reflectance standards, between a) 400 nm to 2400 nm, and enlarged in the spectral range between: b) 800 nm and 1200 nm; c) 1600 nm and 2000 nm.

The steps are a common problem when viewing targets with porous surfaces (non-uniform target geometry) such as grass and soils, and targets measured at closer distances can have the steps because each optic fiber in the bundle of the bare input sees a slightly different spot due to slight offsets of the fiber‟s FOV. Those effects can be reduced or even eliminated by rotating the sample (D. Campbell, personal communication, 07 June 2006); this procedure was adopted for all measurements. But because the first target was a single uniform leaf (spectra represented on Figure 3.3) and moreover, not all spectra (Figure 3.3 and 5) presented the steps, we believe the problem can not be exclusively related to the target or the fiber optic.

The tiles tested differ in the type of finishing, and according to Springsteen (1999) the smooth finish that is given to some tiles to improve their durability has a significant specular component, which can affect the measurements. The Asia Tile *(5)* was very glossy (e.g. very smooth and shiny) the Niro Whites *(6)* and Tau Ceramica *(8)* were glossy, the Nordico Snow *(11)* was slightly glossy, and the Milenio Blanco *(9)* and San Lorenzo *(10)* were dull. It was observed that the steps at splice points occurred when using the glossy tiles (with high specular component). And this observation was reaffirmed by sanding part of the Asia Tile *(5)* which reduced the size of the steps at the splice points on the reflectance spectra of a leaf. So the Asia Tile *(5)*, Niro Whites *(6)*, Tau Ceramica *(8)* and the Nordico Snow *(11)* were considered unsuitable as reflectance standards to be used with the CAPP.

Although the barium sulphate *(2* and *3)* presented reasonable results, it proved unsuitable for field conditions. It was easily rubbed off the dish on to which it was pressed, and it was impossible to clean contaminating dust from its surface. To make the barium sulphate more resistant Knighton and Bugbee (2004) mixed it with white latex paint, but the mixture caused a decrease in the reflectance. Observing the reflectance factor of the Dulux weathershield white paint *(4)* tested in this study (Figure 3.2b), it is predictable that if mixed with barium sulphate, the resultant mixture will also have a decrease in the calculated reflectance spectra, and even worse, this decrease will not be equal in all wavelengths.

In summary, the tiles Milenio Blanco *(9)* and San Lorenzo *(10)* were the materials that best fitted the profile of a large, durable and low-cost reflectance standard for use with the CAPP. But because the San Lorenzo Blanco Niveo *(10)* has a higher reflectance than the Milenio Blanco *(9)*, around 80% and 75% respectively (Figure 3.2a), it was the one selected to be used as the reflectance standard for the CAPP. Since the material chosen as a reflectance standard is not a perfectly lambertian reflector (e.g. perfectly reflective and uniformly radiative in all directions), the azimuthal angular effect of the San Lorenzo (*10*) tile was analysed. Using the FieldSpec® Pro FR attached to the CAPP a white reference scan of the San Lorenzo tile was recorded, then the probe was rotated clockwise at 45°, 90°, 135°, 180°, 225°, 270°, 315° and 360° and scans were recorded at each position. The procedure was repeated three times. The mean difference, comparing the first scan (obtained at initial position) to the scans at other positions varied from zero to 1%, a satisfactory result. Subsequently, it has been used intensively in studies of proximally sensed pasture sward reflectance in different regions of New Zealand.

3.3.2. Selection of reflectance standard tile for further study

The San Lorenzo Blanco Niveo (*10*) tile fulfilled the conditions required of a reflectance standard for the CAPP. Before recommending use of the tile we considered it was important to assess the variability in reflectance that exists in a small sample of these tiles. The reflectances from six San Lorenzo tiles were compared and found to have very small variation (a mean variation of 0.04 for the range 400-2400 nm). Among the six tiles, one was from a different production batch. When this tile was removed, the 5 remaining tiles had even smaller variation in reflectance (a mean variation of 0.02, for the specified spectral range). For the purposes of our further study any of these five San Lorenzo tiles could be used as a backup standard reflectance tiles. We recommend that other researchers requiring large reflectance standards for field use also establish a back up set of reflectance standard tiles.

Also, since the San Lorenzo ceramic tile has a reflectance of 80%, a lower SNR is expected if compared to a near 100% reflecting reflectance standard (e.g. spectralon®) under the same illumination conditions. Thus, depending on the application and SNR required, the illumination source should be powered to compensate for this characteristic. For our application (acquiring reflectance factor of pasture swards in the field) an ASD 50 Watt tungsten-quartz-halogen lamp (light mounted on the top of the CAPP) is used; the acquired pasture spectra present some noise at the far end of the mid-infrared because of low SNR, but that can be reduced using a smoothing filter in data pre-processing.

It is important to mention that when collecting reflectance factor data, the viewing geometries must be taken into account, since even the best reflectance standard will never be a perfect reflecting diffuser. When varying the angle of the fiber optic input on the CAPP the steps at splice points varied in magnitude. The results showed above were obtained with the viewing geometry that minimized the steps (light source at zenith and fiber optic at 18 degrees from zenith) taking into account the limitation imposed by the CAPP structure and FOV of the instrument. Consequently the results presented in this study may vary when using other probes with different viewing geometries.

It is also relevant to point out that the decision to run the first tests using the ASD plant probe, although not ideal, was the only option available to compare the spectralon® with the other materials tested as reflectance standard. Since the geometry and construction materials of the plant probe were designed to minimize measurement errors, it was assumed if a material did not perform well with the plant probe (looking at single leaf) it would be also not suitable for the CAPP (looking at complex canopies).

3.4. Conclusions

A matt white ceramic tile (San Lorenzo Blanco Niveo) has been proven suitable as a large, durable and low cost reflectance standard to obtain field pasture reflectance spectra over the range of 400-2400 nm, using an ASD FieldSpec® Pro FR spectroradiometer attached to the CAPP. Although the presented study was focused to find a reflectance standard for a specific usage (with the CAPP), the white ceramic tile San Lorenzo Blanco Niveo seems to be a reasonable option of reflectance standard for use in other applications in both laboratory and field conditions. It is necessary to stress that spectral curves obtained with San Lorenzo tile should not be compared to those obtained with any other reference panels, since each reference has different properties.

CHAPTER 4:

Influence of surface moisture on field hyperspectral data from pasture canopies

Abstract

An ASD FieldSpec® Pro FR coupled to the CAPP (canopy pasture probe) was used to obtain reflectance factor spectra of pastures under three treatments *dry*, *damp* and *wet*. The research objective was to examine the influence of surface moisture on hyperspectral measurements obtained in the field under artificial illumination, at wavelengths ranging from 400 to 2468 nm. Overall, the reflectance factor sensitivity to canopy wetness increased with longer wavelengths, the maximum peaks were at 1450, 1920 and 2420 nm. The analyses used reflectance factor, first derivative and continuum removed spectra. Statistically significant differences were observed for mean spectral data between the three treatments for several parts of the infrared (IR) region; however no significant differences were obtained at visible (Vis) wavelengths. The impact of surface moisture on pasture quality prediction was also investigated, the regression results showed the pasture wetness influenced the crude protein prediction, especially at infrared wavelengths; in general lower correlations and higher RMSECVs were obtained for the *wet* samples comparing to *dry* and *damp* samples.

Keywords: wetness; pasture canopies; hyperspectral data; quality prediction

4.1. Introduction

The economy of New Zealand is extremely dependent on the grazed pasture systems. Fifty four million tonnes of pasture dry matter is consumed each year. Twenty two million tonnes are consumed by sheep, 19 million tonnes by dairy cattle, 11 million tonnes by beef cattle and 2 million tonnes by deer (MFE 2007). For efficient feeding of livestock, both pasture quantity and quality need to be regularly monitored. Currently the assessment of pasture bulk quality is conducted by laboratory near infrared (NIR) spectral analysis of dried samples of the sward (e.g. Corson *et al.* 1999) at more affordable prices and faster than the traditional wet chemistry method, however the samples still need to be clipped and transported to the laboratory. Remote and proximal sensing of Vis-NIR diffuse reflectance potentially offers a rapid on the go *in situ* measurement of pasture quality (e.g. Mutanga *et al.* 2004; Sanches *et al*. 2006 and Thulin *et al.* 2006).

Accurate spectral data using natural illumination can only be acquired under high sun elevations, cloud-free sky, and minimal atmospheric turbidity (Williams and Woody Jr 1987). Windy, changeable weather (Garnier 1958) and high cloud cover (Wardle 1986) in New Zealand and other mid-latitude countries limit the opportunities to use remote diffuse reflectance sensing technologies for reporting on pasture quality.

To address this issue on the field scale, the Canopy Pasture Probe (CAPP) was developed (Chapter 2) to acquire proximal sensed diffuse reflectance factor spectra from pastures. The CAPP minimizes the effects of unwanted contributions to the acquired spectra by providing controlled illumination, a condition often obtained only in the laboratory. The CAPP allows the spectral measurements to be done *in situ*, at canopy scale.

The CAPP has been used to acquire spectral data to evaluate calibrations for the prediction of pasture quantity and quality parameters (Sanches *et al.* 2006, Sanches *et al.* 2008, Betteridge *et al.* 2008). It has been proven very efficient in controlling the illumination, and also as a wind barrier keeping the target still during the measurements. Unfortunately not all atmospheric influences can be controlled under field conditions, and although the sunlight and wind are not a concern when using the CAPP, the target surface moisture can be. Thus it is essential to have knowledge about the effect of wetness upon hyperspectral data collected from pasture.

Besides the rainfall, in cool temperate climates pasture is frequently found to be wet due to dewfall, distillation and guttation (Hughes and Brimblecombe 1994). Both dewfall (the flux of water vapour from air to surface) and distillation (the flux of water vapour from soil to canopy) are subdivisions of dew (Monteith 1957); while guttation is the fluid exuded from plant tissue through leaf pores (Richards 2004).

There are two kinds of water effect on vegetation reflectance spectra, the ones that can be explained solely by water radiative properties - primary effects, and others that can not be exclusively explained by these properties - secondary effects. Overall the primary effects are more prominent than the secondary ones, and within the 400-2500 nm spectrum the most significant influence of water content is the direct absorption of radiation by water; it causes the reflectance to decrease as the leaf water content increases, particularly in the 1300-2500 nm range where the water absorption is strong (Carter 1991).

According to Harris *et al*. (2005), the increase in surface moisture reduces reflectance in the Vis, NIR and SWIR regions of the electromagnetic spectrum and also significantly changes the overall shape of the spectra. Besides, the dew wetness can mask some important spectral features; Pinter (1986) observed that the actual reflectance differences existing between cultivars, growth stages and other factors affecting productivity of wheat were masked by dew wetness.

In this context, the objective of this paper was to examine the effect of surface moisture of pasture canopies on spectral data obtained in field conditions under artificial illumination. The spectral quantity analysed was the reflectance factor, plus two spectral transformations, first derivative and continuum removal. The impact of wetness on pasture quality prediction was also analysed.

4.2. Material and methods

4.2.1. The study area

The study area is located in Hamilton, New Zealand (Latitude: 37° 46“ S and longitude: 175° 18“ E). In this region the average rainfall per month varies from around 70 mm (summer) to 130 mm (winter) and mean monthly temperature ranges from 9°C to 19°C (NIWA 2007). The soil is classified as an Allophanic soil (Hewitt 1993).

For this study four paddocks of varying pasture quality were selected. In each paddock five 50 x 40 cm quadrats were selected to be measured. To analyse the effect of wetness on the spectra three treatments were applied: *dry* – no water was added to the plots; *damp* – the 0.2 m^2 quadrats were sprayed (misted) with 33 ml of water; and *wet* – the quadrats were misted with an additional 100 ml of water. Consequently, the 33 ml and 100 ml of water applied on the plots correspond to 165 ml m^{-2} (0.165 mm) and 500 ml m^{-2} (0.50 mm) respectively.

4.2.2. Field canopy reflectance factor measurements and chemical analysis

The spectral data were collected in the field using an ASD FieldSpec® Pro FR attached to the CAPP (Chapter 2) (Figure 4.1). A white ceramic tile was used as a reflectance standard (Sanches *et al*. 2009, Chapter 3). The measurements were taken in mid January 2007.

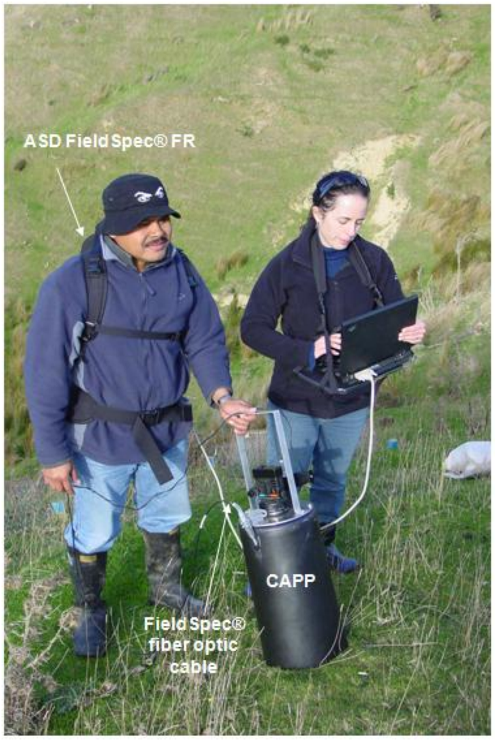

Figure 4.1. Collection of canopy pasture spectral data using the CAPP attached to an ASD FieldSpec® Pro FR.

The reflectance factor was recorded from each one of the 20 quadrats (five quadrats in each of the four paddocks) under each of the three wetness conditions (*dry*, *damp* and *wet* treatments). Ten spectra were acquired per quadrat and averaged into one spectrum in data pre-processing. This procedure made it possible to represent the grass in the entire plot. Initiation of a measurement involved scanning the white reference, then recording the 10 reflectance factor spectra of the quadrat under dry conditions (*dry* spectra) before misting the plot with 33 ml of water prior to taking another 10 reflectance factor spectra (*damp* spectra), then finally misting the plot with an additional 100 ml of water and recording the final 10 reflectance factor spectra (*wet* spectra). The interval between misting and recording the reflectance factor was around 3 minutes. Every plot was also photographed. With field reflectance factor measurements completed the quadrat was clipped and dried pasture was analysed for crude protein content by near-infrared reflectance spectroscopy - NIRS (Corson *et al.* 1999) at the FeedTECH laboratory based at AgResearch in Palmerston North, New Zealand. The dry matter (kg/ha) and botanical composition (dead material, green grass leaf, clover, weeds and seed heads) were also determined for each plot.

4.2.3 Data pre-processing and data analysis

The pre-processing of the spectral data consisted of averaging and smoothing. The 10 spectra measured for each sample were averaged to form a single spectrum, this procedure was adopted to minimize the noise (ASD 2000) and also to better represent the total area of the plots (0.2 m^2). Next the spectra were smoothed with a Savitzky-Golay filter (Savitzky and Golay 1964), using 81 smoothing points and polynomial order 4.

Spectral transformations have been used intensively in an effort to eliminate or reduce effects not related to the target‟s properties of interest and enhance spectral features. After pre-processing, two transformations were applied to the spectral data: derivative and continuum removal. The derivative of a spectrum is the rate of reflectance change with respect to wavelength (Demetriades-Shah *et al.* 1990). This technique is very promising for use with hyperspectral remote sensing data, and first and second order derivatives have been the most common (Tsai and Philpot 1998). In this study, the first derivative was employed. Continuum removal was applied to the major absorption features, localized between 425-518 nm, 550-750 nm, 910-1081 nm, 1116-1284 nm, 1285-1666 nm and 1796-2214 nm. This transformation consists of an estimate of the other absorption features present in the spectrum, not including the one of interest (Clark and Roush 1984). The continuum is a convex hull fitted over the top of a spectrum using straight-line segments that connect local spectra maxima; it is removed by dividing the reflectance value by the convex hull (Schmidt and Skidmore 2001). It normalizes reflectance spectra in order to allow comparison of individual absorption features from a common baseline (Kokaly 2001).

To ascertain if there was a significant difference in mean spectra between the three treatments a *t*-test was applied, for each wavelength, on the reflectance factor, first derivative and continuum removed data. The null hypothesis was H_o: $\mu_{1i} = \mu_{2i}$ versus the alternate hypothesis H_a: $\mu_{1i} \neq \mu_{2i}$ where: μ_{1i} and μ_{2i} are the mean spectral values (of reflectance factor, first derivative and continuum removed values) at wavelength *i*, between each pair of *dry*, *damp* and *wet* treatments.

To evaluate the pasture wetness impact on the overall quality of crude protein prediction, partial least squares regressions (PLSR) (Geladi and Kowalski 1986) were carried out between the measured crude protein content of the plots and the reflectance factor, first derivative and continuum removed spectra, at VIS-IR, Vis and IR wavelengths, for each treatment. Full cross-validation was used as the validation method. The goal here was not to evaluate the prediction by itself, but to compare the results (correlation and root mean square error) obtained for each treatment.

4.3. Results

4.3.1. Pasture samples

The pasture samples collected from the 20 plots (Figure 4.2) analysed in this experiment varied between 9-22% of crude protein; 2122-4099 kg/ha of standing harvestable dry matter (DM) comprised of 7-50% of dead matter; 27-72% of green grass leaf; 0-32% of clover; 0-25% of weeds; and 0-23% of seed heads.

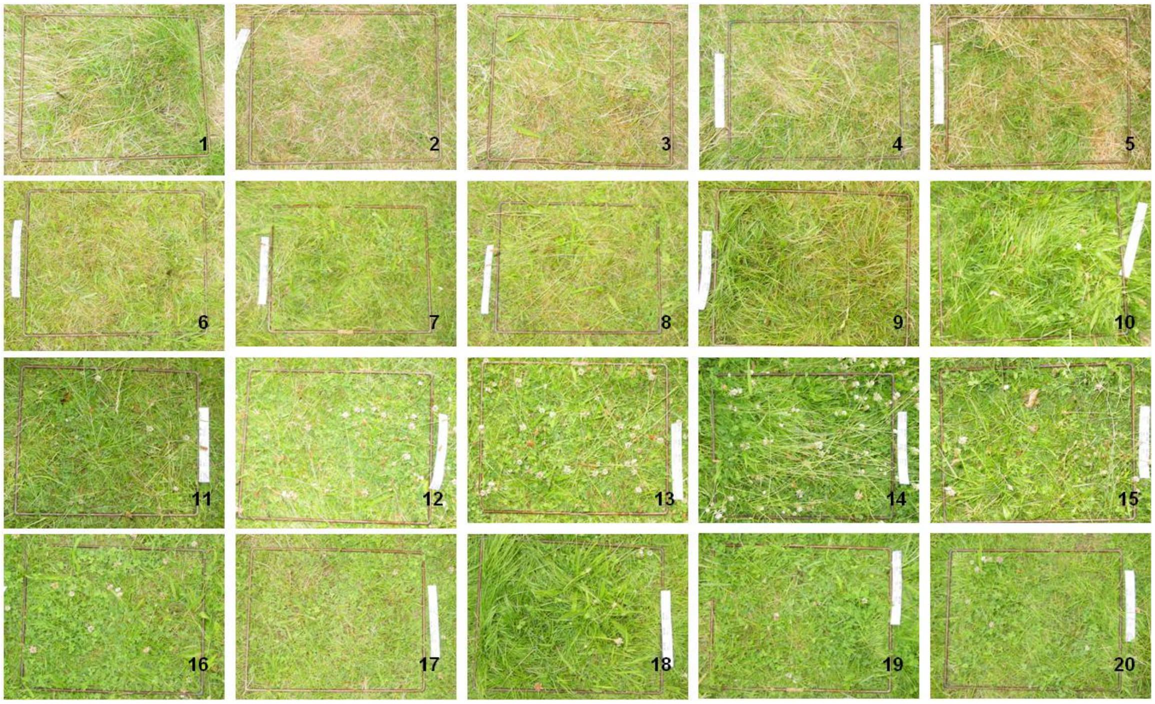

Figure 4.2. Photographs of pasture plots.

4.3.2. Reflectance factor data

In this paper the visible (Vis) refers to the wavelengths between 400-700 nm, the term near-infrared (NIR) refers to the range 700-1300 nm, and mid-infrared (MIR) to 1300-2500 nm; these divisions are common in the remote sensing literature, but sometimes NIR is applied for the whole range from 700 to 2500 nm.

The differences in reflectance factor spectra among treatments varied along with the plots. In general, at Vis wavelengths hardly any changes were observed visually between treatments. In the NIR, as more water was misted over the pasture canopies two responses were detected: a decrease in reflectance factor values at all NIR (Figure 4.3b) and; an increase in reflectance factor for shorter NIR (< 970 nm) after which the reflectance factor decreased, followed by a reflectance factor augment towards 1100 nm and decreasing beyond this wavelength (Figure 4.3a). In the MIR the reflectance factor for all samples decreased with increase in surface moisture.

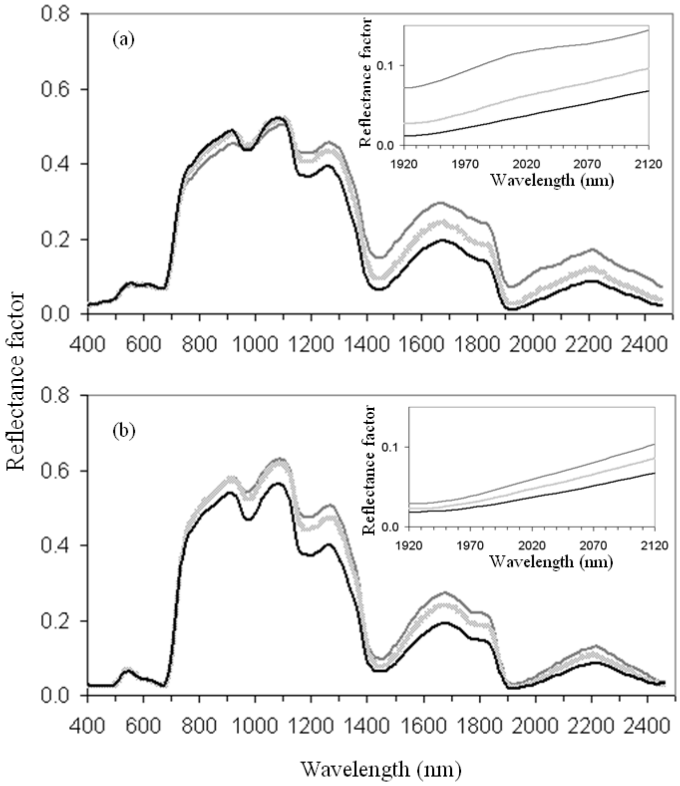

Figure 4.3. Response of pasture canopy reflectance factor to the treatments *dry* (dark grey line), *damp* (light grey line) and *wet* (black line) for pasture plot 3 (a) and plot 7 (b). Overall hardly any change was observed at Vis; at NIR two reactions were noticed: (a) both increase and decrease in reflectance factor values, and (b) decrease in reflectance factor values at all NIR; at all MIR wavelengths the reflectance factor decreased.

For a better representation of the effect of surface wetness on reflectance factor spectra, the reflectance factor difference was calculated by subtracting the *dry* reflectance factor of a pasture sample from the *damp* reflectance factor of the same pasture sample; and by subtracting the *dry* reflectance factor of a pasture sample from the *wet* reflectance factor of the same sample. For *damp* (Figure 4.4a) and *wet* (Figure 4.4b) treatments the reflectance factor differences ranged, in absolute values, between 0-6.7% and 0-11.2%, respectively. The largest reflectance factor differences were observed throughout the infrared (IR), and only small changes were observed in the Vis.

Two distinct behaviours were detected in the reflectance factor difference data, dividing the pasture samples into two groups. To improve visualization, the reflectance factor differences of all the samples from each group were averaged, and these are illustrated in Figures 4.4c and 4.4d. The first group showed a slight dip in reflectance factor difference near 530-560 nm and another dip around 690-760 nm, conversely the second group showed a slight peak and an increase for the same wavelengths ranges. Between 1920-2210 nm both groups showed an increase in reflectance factor difference, but for the first group the increase was not linear; the two absorption bands at around 1940 nm and 2060 nm (that looks like a little peak between the wavelengths 1970-2070 nm) observed on the *dry* reflectance factor spectrum (zoom in on Figure 4.3a) was not present at *damp* and *wet* spectra. In each group the same trend in reflectance factor difference was presented by both *damp* and *wet* data, and although different in magnitude, the location of major peaks and dips occurred at similar wavelengths.

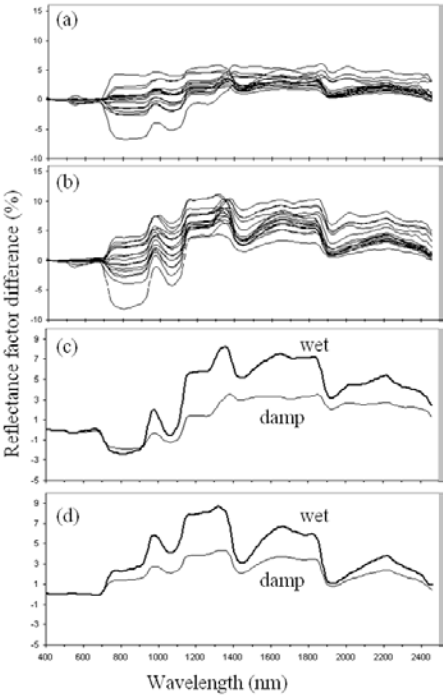

Figure 4.4. Percentage of reflectance factor difference for all samples (a) under *damp* treatment and (b) under *wet* treatment; and samples divided into two groups (c) average of 1st group under *damp* and *wet* treatment; (d) average of 2nd group under *damp* and *wet* treatment. The reflectance factor difference was calculated by subtracting the *dry* reflectance factor of a pasture sample from the *damp* reflectance factor of the same pasture and from the *wet* reflectance factor of the same sample.

To enhance the differences between treatments the reflectance factor difference was found to be very effective. However to identify at which wavelengths the reflectance factor was more affected by the pasture canopy wetness, the reflectance factor sensitivity (adapted from Carter 1991) was used. It was calculated by dividing the reflectance factor difference (for both *damp* and *wet* samples) by the *dry* reflectance factor. All samples presented the same behaviour, for both *damp* and *wet* treatments (Figures 4.5a and 4.5b). The variation in sensitivity magnitude among samples occurred mostly at maximum peaks, observed at 1450 nm, 1920 nm and 2420 nm. In general, the reflectance factor sensitivity to surface moisture showed a tendency to increase with the increase in wavelength; this is visualized better in the averaged data (Figure 4.5c).

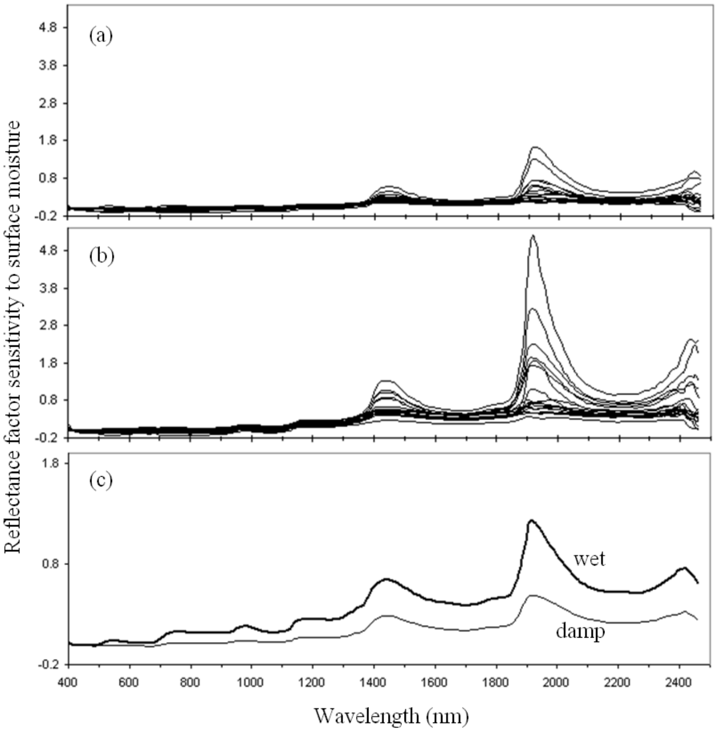

Figure 4.5. Reflectance factor sensitivity to surface moisture for all (a) *damp* data, (b) *dew* data, and (c) averaged data in a zoom in scale. The reflectance factor sensitivity was calculated by dividing the reflectance factor difference by the *dry* reflectance factor of each sample, for *damp* (grey line) and *wet* (black line) data.

4.3.3. T-test results

The three treatments were clearly differentiated visually in the pasture reflectance factor spectra only at MIR (Figure 4.6a). According to the *t*-test, between 400-2468 nm, the differences between treatments were statistically significant from 1296 nm for *dry* versus *damp*; from 1134 nm for *damp* versus *wet*; and between 959-1012 nm and from 1121 nm for *dry* versus *wet* until the end of the spectra analysed (Figure 4.6b).

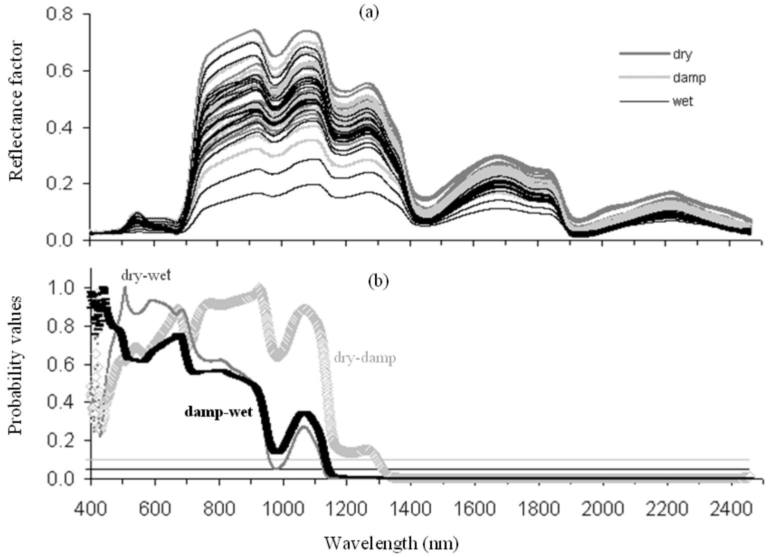

Figure 4.6. (a) Reflectance factor spectra for the 20 plots analysed. And (b) results of *t*-test for the reflectance factor data. Three classes were considered: *dry* versus *damp*, *damp* versus *wet*, and *dry* versus *wet*. The dots plotted show the probability value when testing if the reflectance factors between classes are significantly different. Horizontal line in black and grey show 95% ($p < 0.05$) and 90% ($p < 0.10$) confidence limits correspondingly.

Because it was very hard to visualize the effect of treatments in the graph of all derivative spectra, the averaged values of the 20 samples per treatment were plotted instead (Figure 4.7a). Statistically significant differences, although not continuous, can be seen in several parts of the IR, beginning from 925 nm for *dry* versus *damp*, from 907 nm for *damp* versus *wet*, and from 821 nm for *dry* versus *wet* class (Figure 4.7b).

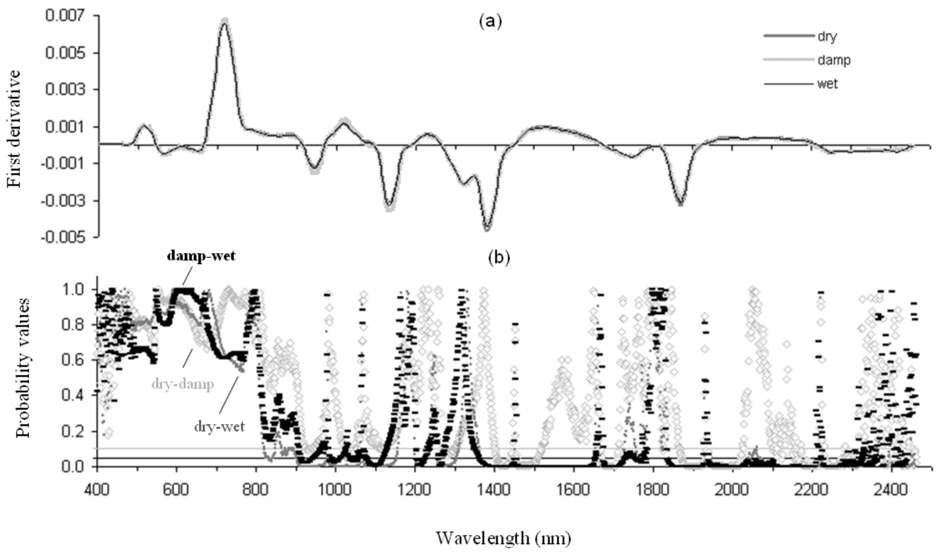

Figure 4.7. (a) First derivative spectra averaged per treatment. And (b) results of *t*-test for the first derivative data of 20 plots. Three classes were considered: *dry* versus *damp*, *damp* versus *wet*, and *dry* versus *wet*. The dots plotted show the probability value when testing if the reflectance factors between classes are significantly different. Horizontal line in black and grey show 95% ($p < 0.05$) and 90% ($p < 0.10$) confidence limits correspondingly.

Based on the continuum removal technique, the treatments were hard to differentiate visually (Figure 4.8a) in the two Vis absorption bands, 425-518 nm and 550-750 nm, but were clearly distinguishable from each other in the four IR absorption bands (910-1081 nm, 1116-1284 nm, 1285-1666 nm, 1796-2214 nm). The *t*-test results agreed with the visual assessment; the differences between treatments were not statistically significant in the Vis absorption bands, but were statistically significant in the IR absorption bands (Figure 4.8b). The exact wavelength intervals where the differences between treatments were statistically significant are shown in Table 4.1.

Table 4.1. Wavelength intervals where the differences between wetness treatments were statistically significant (*t*-test) in the continuum removed spectra.

Absorption band	*dry* versus *damp*	*dry* versus *wet*	*damp* versus *wet*
910-1081 nm	920-1058 nm	911-1063 nm and 1072-1080 nm	912-1059 nm and 1073-1080 nm
1116-1284 nm	1117-1271 nm	1117-1283 nm	1117-1283 nm
1285-1666 nm	1286-1665 nm	1286-1665 nm	1286-1422 nm and 1513-1665 nm
1796-2214 nm	1836-2156 nm	1832-2144 nm	1836-1865 nm

Considering that the first and last wavelengths on each continuum removed band (endpoints of the continua) always have value 1; the *dry* versus *wet* and *damp* versus *wet* classes were statistically different for the whole absorption band 1116-1284 nm, and the *dry* versus *damp* and *dry* versus *wet* for the entire band 1285-1666 nm.

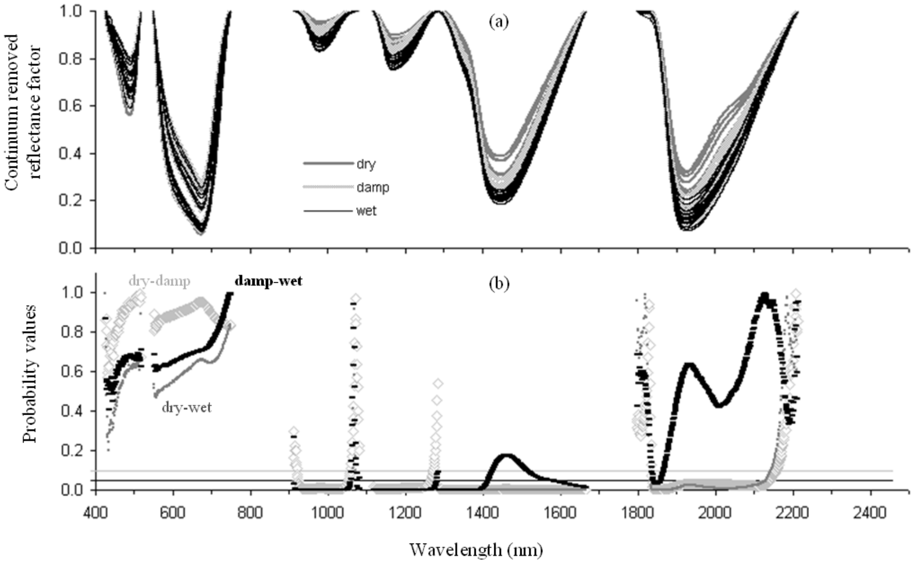

Figure 4.8. (a) Continuum removed reflectance factor spectra for the 20 plots analysed. And (b) results of *t*-test for the continuum removed data. Three classes were considered: *dry* versus *damp*, *damp* versus *wet*, and *dry* versus *wet*. The dots plotted show the probability value when testing if the reflectance factors between classes are significantly different. Horizontal line in black and grey show 95% ($p < 0.05$) and 90% ($p < 0.10$) confidence limits correspondingly.

4.3.4. Pasture crude protein

The results for PLSR between the measured crude protein content of the plots and the reflectance factor, first derivative and continuum removed spectra at Vis-IR, Vis and IR wavelengths, for *dry*, *damp* and *wet* samples are shown in Table 4.2. Because cross-validation was used, the root mean square error was called RMSE of cross-validation (RMSECV). The correlation and RMSECV values per se should be overlooked in this case because the method of prediction is not being evaluated; mainly because of an insufficient dataset size and (crude protein) range. The intention was only to compare the regression results of the *dry*, *damp* and *wet* samples, i.e. to measure the impact of pasture wetness in the crude protein prediction. Overall when comparing treatments, the highest correlations and lowest RMSECVs were obtained for the *dry* samples and lowest correlations and highest RMSECVs for *wet* samples. For regressions combining Vis-IR or using only Vis wavelengths the results for *dry* and *damp* treatments were very similar for first derivative and continuum removed data.

Table 4.2. Correlation and root mean square errors of cross-validation (RMSECV) of PLSR carried out between measured crude protein and reflectance factor, first derivative and continuum removed spectra, at Vis-IR, Vis and IR wavelength ranges, for *dry*, *damp* and *wet* samples. For reflectance factor and first derivative data the Vis correspond to wavelengths between 400-700 nm and IR to 700-2468 nm; for continuum removed spectra the Vis correspond to absorption bands between 425-518 nm and 550-750 nm, and IR to 910-1081 nm, 1116-1284 nm, 1285-1666 nm and 1796-2214 nm.

	Spectral range	*dry* treatment		*damp* treatment		*wet* treatment	
	analysed	Correlation	RMSECV	Correlation	RMSECV	Correlation	RMSECV
Reflectance factor	Vis-IR	0.76	0.34	0.69	0.39	0.68	0.40
	Vis	0.85	0.27	0.80	0.32	0.68	0.40
	IR	0.63	0.41	0.59	0.43	0.58	0.43
First derivative	Vis-IR	0.79	0.31	0.78	0.32	0.76	0.34
	Vis	0.85	0.27	0.82	0.30	0.76	0.34
	IR	0.74	0.37	0.69	0.39	0.65	0.40
Continuum removed	Vis-IR	0.75	0.34	0.74	0.35	0.60	0.45
	Vis	0.78	0.33	0.77	0.33	0.72	0.37
	IR	0.67	0.38	0.52	0.45	-0.08	0.66

4.4. Discussion

Even though the dataset collected for this experiment was not large, there was reasonable range in pasture characteristics, and sufficient diversity in canopy structure (Figure 4.2) to result in considerable variance in the reflectance factor spectra. Although the effect differed, all plots had their reflectance factor spectra affected in some way by the water misted on the pasture canopies.

In contrast to our results, Pinter (1986) reported that moderate levels of natural dew resulted in a marked increases in the reflectance of wheat canopies at Vis wavelengths, decreases between 1150 and 2350 nm (MIR), and no change at NIR wavelengths. Madeira *et al.* (2001) reported similar results for dew on planophile bentgrass canopies, with an increase in Vis reflectance and a decrease on MIR, although a slight decrease was observed in the NIR. For erectophile bluegrass, however, the most noticeable change caused by dew was a decrease in reflectance in the MIR, followed by a decrease in the NIR, while the reflectance in Vis was almost unaffected. Our results of misted water on pasture canopies are in accordance with those reported for erectophile bluegrass, although for some plots an increase in reflectance factor, instead of a decrease, was observed in the NIR.

To better comprehend the effect of wetness on the pasture reflectance factor spectra, it is necessary to keep the mechanism of vegetation reflectance in mind. The energy incident on a leaf/canopy can be reflected, absorbed or transmitted, and because of the strong absorption by leaf pigments in the Vis, and by water in the IR beyond 1300, the reflectance in these wavelengths is, respectively, very low and relatively low; while in the IR from 700 to 1300 nm the reflectance is relatively high, due to internal leaf scattering and nearly zero absorption (Knipling 1970).

In the spectral range 400-2500 nm, the most significant influence of water content on leaf reflectance is the direct absorption of radiation by water (primary effect), that causes the reflectance to decrease as the leaf water content increases, particularly in the 1300-2500 nm range where the water absorption is strong. And although the primary effects are overall more prominent, transmissive properties of water can also influence the reflectance of leaves (secondary effects) (Carter 1991). That was observed for bean leaves infiltrated with water: due to the leaf air cavities having been filled with water, many of the refractive index differences within the leaf were eliminated causing a drastic reduction in IR reflectance (Knipling 1970). The influence of water on leaf reflectance spectra is not the same for all plants, for example with a reduction of relative water content from 97 to 77%, the reflectance at 540 nm was considerably increased on maize leaves, remained about the same on soybeans leaves, and slightly decreased on cotton leaves (Woolley 1971). These differences probably reflect changes in the intercellular air spaces of the leaf.

In the present study, with the increase in surface wetness, two opposite behaviours were also observed near 540 nm (530-560 nm), a slight increase in reflectance factor for one group of the samples and a decrease for the other group. All pasture plots analysed had different combinations of grass, legume and weeds; the first group, on average, contained smaller percentages of grass, legume and dry matter, and a greater percentage of dead matter, compared to the second group. In addition, with the increase in water, the first group of samples had an increase in reflectance factor in the red-infrared transition region (690-760 nm), while a decrease was observed for the second group. The increase in leaf reflectance in this region has been attributed to the reduction of chlorophyll absorption in response to a change in environmental conditions (Carter 1991).

For vegetation, in the range 400-2500 nm, the central wavelengths of the water absorption bands are near 970, 1200, 1450 and 1940 nm (Curran 1989). In general the water content of green leaves quickly saturates and dominates the reflectance spectra at those wavelengths (Elvidge 1990). In our experiment the maximum reflectance factor sensitivity to pasture canopy surface moisture, for the range analysed (400-2468 nm), occurred at 1450 nm, 1920 nm and 2420 nm. Smaller, peaks at 970 and 1200 nm were also observed (Figure 4.5c). Maximum peaks of reflectance sensitivity to relative water content of leaves at 1450 and 1920 nm have been reported previously, along with reflectance at 2500 nm (Carter 1991).

The changes in vegetation reflectance mentioned above occur only after the radiation has entered into the leaves, but that is not the only way the reflectance spectrum is affected by water. Water drops can act as lenses sending back a larger fraction of the incident light in the original direction, making targets like moist grass strongly retroreflective (Fraser 1994). This great increase in vegetation canopies" reflectance due to the high specular reflection of droplets has been reported by other authors (Pinter 1986 and Madeira *et al.* 2001), however this phenomenon was not observed in our experiment.

4.4.1. Significant differences between treatments

In the present study, the *t*-test applied to the reflectance factor, first derivative and continuum removed mean data showed no significant effect of pasture wetness for Vis wavelengths. However, for the IR region the result was more complex and dependent on the spectral data analysed. In the NIR and beginning of MIR, for the two transformed datasets, the differences between wetness treatments were significant at various wavelengths (Figures 4.7b and 4.8b), while with the reflectance factor data significant differences ($p < 0.10$) were obtained only between 958-1012 nm for the *dry* versus *wet* class (Figure 4.6b). At remaining MIR wavelengths, significant differences ($p < 0.05$) were obtained for a range of wavelengths for all three spectral datasets.

At the wavelengths corresponding to the water absorption bands, 165 ml m^{-2} of water misted on the plots was enough to significantly influence the spectral data at the water bands around 1450 and 1920 nm (maximum sensitivity); but the bands near 970 and 1200 nm were only affected after 500 ml m^{-2} water was applied. Continuum removed data allowed the difference between *dry* and *damp* treatments to be detected statistically at all four water absorption bands mentioned (Figure 4.8b).

4.4.2. Pasture crude protein prediction

The pasture quality needs to be monitored if feed supply to grazing animals is to be optimized. Traditionally, field pasture samples are clipped and sent to a laboratory to be analysed using wet chemistry techniques, which can be expensive. More recently, laboratory near infrared reflectance spectroscopy (NIRS) has been used in New Zealand to estimate forage quality at more affordable prices (Corson *et al.* 1999). The next step, already being investigated, is to predict quality parameters using hyperspectral data *in situ* (Mutanga *et al.* 2004, Christensen *et al.* 2004, Sanches *et al.* 2006, Thulin *et al.* 2006). However, that will only become reality if accurate estimations can be obtained, and these depend upon the collection of high quality field spectral data for calibration and prediction steps.

The measurement of crude protein provides an important indication of pasture quality. As demonstrated in this paper, the correlation between reflectance factor data and pasture quality was compromised by the surface wetness of pasture canopies, reducing the performance of crude protein prediction. When the reflectance factor spectra were transformed by applying first derivative and continuum removal the regression results were less affected by the surface moisture, with exception of the prediction analyses for *wet* data using the continuum removed spectra in the Vis-IR and IR range.

Twelve absorption features have been related to foliar protein content in the range 400-2400 nm, with three of them found at 1940, 1980 and 2060 nm (Curran 1989). Among the pastures samples analysed, the crude protein ranged between 9-22%, and it was observed on the *dry* reflectance factor spectra that, between 1920-2210 nm, all the samples with crude protein values equal to or greater than 14% showed a single absorption feature (Figure 4.3b), while all the samples with crude protein lower then 14% had two smaller absorption features (Figure 4.3a). These differences were better highlighted in the *dry* continuum removed reflectance factor between 1796-2214 nm, where the samples with lower crude protein not only presented a slight bump around 2000 nm, they also had smaller band depths. However, for *damp* and *wet* spectra this differentiation was not possible, because the water had masked the absorption features.

4.5. Conclusion

The development of devices like the CAPP is very useful when working with remote sensing techniques in field conditions, especially in places of changeable illumination conditions like New Zealand. However there are other challenges for the use of spectroscopy to predict pasture quality parameters *in situ*, and as demonstrated in this research the surface wetness of pasture canopy is definitely one variable that needs to be taken into account. The ideal would be to collect spectral data without any surface moisture in the pasture, but in the real world that might be unfeasible; or to have robust models that effectively remove the effect of water from the sensed spectra. Nevertheless, some straightforward approaches to deal with pasture surface moisture when predicting quality would be to avoid collecting spectral data at least when the pasture is very wet (e.g. after rain) and to use mathematically transformed spectra (such as derivatives and continuum removal).

CHAPTER 5:

Proximal sensing the botanical composition of New Zealand dairy and sheep pastures

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Some results of this study have been orally presented in the 11th Australasian Symposium on Precision Agriculture, 14th September 2007, Massey University, Palmerston North, New Zealand; and a summary was published online: Sanches, I.D., Tuohy, M., Hedley, M., Mackay, A. (2008) Proximal sensing pasture composition. *Southern Precision Agriculture Association: Precision Ag News*, 17. Available online at (http://spaa.com.au/files/catalog/march_08(proximal_sensing_pasture_composition).pdf).

Abstract

Information on botanical composition provides insights into the productivity, quality, weed prevalence and persistence of sown species in perennial grass-legume based pastures. In New Zealand pastures are established using a combination of perennial ryegrass (*Lolium perenne*) and white clover (*Trifolium repens*) species. Often the sown species do not persist allowing ingress of unwanted species (weeds). Pasture composition also changes with season, due to variation in temperature, day length and rainfall and with grazing management. All these factors impact on feed quantity and quality. Apart from visual observation, current options for assessing the botanical composition of pasture are labour-intensive and time-consuming and hence have limited utility on-farm. In this paper (Chapter) the potential to predict the botanical composition (expressed as proportions of grass, legume and weed) of New Zealand dairy and sheep pastures from their proximally sensed spectral reflectance is tested. The PLSR models predicting either the legume, grass or weed content of hill pastures grazed by sheep were inaccurate (legume: R^2 = 0.02-0.39, SD/RMSECV = 1.0-1.2, grass: R^2 = 0.02-0.44, SD/RMSECV = 0.9-1.3, and weed: R^2 = 0.00-0.33, SD/RMSECV = 0.9-1.0), whereas more accurate predictions were obtained for the legume (R^2 = 0.19-0.74, SD/RMSECV = 1.0-1.9) and grass (R^2 = 0.02-0.85, SD/RMSECV = 1.0-2.5) components in lowland dairy pasture. Predictions of the botanical composition of autumn samples from sheep and dairy pastures combined yielded reasonable accuracy when predicting grass (R^2 = 0.81, SD/RMSECV = 2.3) and legume (R^2 = 0.80, SD/RMSECV = 2.2) proportions. The inaccurate predictions might be explained by the fact that the diversity found in the field and observed in the pasture spectral data were not taken into account in the pasture botanical separation.

Keywords: botanical composition, pasture, field spectroscopy, grass-legume swards.

5.1. Introduction

Grassland ecosystems play the major role in the New Zealand economy, with dairy, beef and sheep grazing systems occupying 12 million ha (Hodgson *et al.* 2005). New Zealand produces 38 percent of the milk products traded internationally, with the dairy industry still dependent on grazed pasture as the main source of feed for the milking cow, despite greater use of supplementary feeds in the last 10 years (Holmes *et al.* 2002).

Perennial pasture species are preferable to annual species in New Zealand because of the large costs associated with re-grassing and the tillage constraints imposed by the rolling and hill land topography. The number of species in a perennial pasture can vary between two and twenty, but in most situations two or three perennial species dominate. Adapted to most parts of the country, tolerant of frequent hard grazing and high treading damage, perennial ryegrass (*Lolium perenne*) and white clover (*Trifolium repens*) are the most frequently sown species and a major component of most lowland swards. On hill and high country farms, where the soil fertility is lower, browntop (*Agrostis capillaris)* and fescue (*Festuca spp.*) are the most common grass species. The grass and legume species complement each other, the grass provides the bulk of herbage, while the legume fixes and supplies nitrogen (N) to the soil and has better nutritive value (Kemp *et al.* 1999b). An advantage of the legume-based pastoral farming system is that the N is provided by biological fixation, reducing the expenses associated with N fertilisers. A key goal of pasture management is to maintain adequate legume percentage in the pasture for sufficient N fixation (Kemp *et al.* 1999a).

Species composition is an indication of pasture quality (Tarr *et al.* 2005). In an ideal pasture the species balance needs to meet the seasonal herbage supply requirements of the grazing animals. Initially, the species sown determine the composition of a pasture, but in time the main determinants are environmental conditions and management practices (Kemp *et al.* 1999b). Pasture normally contains a mix of sown and unsown grass and legume species, and unwanted species. Successful grazing management strategies encourage the persistence of desirable and productive species (Matthews *et al.* 1999). Pasture botanical composition is therefore an important attribute to be able to define and regularly assess for both agronomic and animal production studies (Little and Frensham 1993).

The traditional methods for estimating the botanical composition of a mixed pasture range from cutting a pasture sample and hand-separating the species through to a visual assessment. Other techniques have been developed including levy-point (Levy and Madden 1933), dry-weight-rank (Jones and Hargreaves 1979) and rod-point (Little and Frensham 1993). All these options with the exception of visual assessment or ranking for assessing botanical composition of pasture are used by researchers only, are labour-intensive and time-consuming. Visual assessment while quicker is subjective, and requires very good calibration otherwise lacks accuracy. A less labour-intensive, objective and accurate method would offer up some real advances for measurement of pasture composition for both researchers and practitioners.

Diffuse reflectance spectroradiometry has proven useful for characterising the reflectance of natural surfaces *in situ*, supporting a variety of agricultural applications (Swain and Davis 1978, Milton *et al.* 2007). For vegetation studies, field spectroscopy has been applied to explore the plant spectral response to water content, water stress and phenological changes (Kumar *et al.* 2001), to discriminate species (Vrindts *et al.* 2002, Schmidt and Skidmore 2003, Yamano *et al.* 2003), to quantify biomass (Hansen and Schjoerring 2003) and to assess pasture quality (Mutanga *et al.* 2004, Thulin *et al.* 2006). For pastures the potential advantages are the speed and increased efficiency of analysis that will enable appropriate real-time decisions to be made for grazing management and pasture improvement (Roberts *et al.* 2004). The objective of the study was to evaluate the potential to quantify the botanical composition (grass, legume and weed proportions) of dairy and sheep pastures in New Zealand using field hyperspectral reflectance data.

5.2. Materials and methods

5.2.1. Study area

Five study sites were selected on dairy and sheep grazed pastures in the North Island of New Zealand (Figure 5.1). The Manawahe, Tokoroa and Atiamuri sites are all close to latitude 38° S and Colyton and Alfredton sites to 40° S.

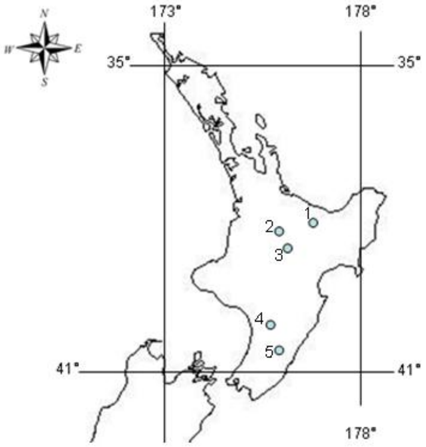

Figure 5.1. Sampling site locations: 1) Manawahe, 2) Tokoroa, 3) Atiamuri, 4) Colyton and 5) Alfredton.

Based upon temperature and rainfall, New Zealand pastures can be divided into six zones: northern, mid central, dry east coast, high-rainfall west coast, high country and cooler southern, but the boundaries of these zones are indistinct and each zone merges with the next (Stewart and Charlton 2006). All study sites are in the mid central zone, characterized by few extremes of temperature and occasionally drought in summer. Pastures are mostly composed of temperate grasses with very few subtropical or C4 grasses, with the hill pastures containing some less productive grasses (such as browntop and Yorkshire fog (*Holcus lanatus*)). The Manawahe site is located in an area very close to the northern zone, which has warmer temperatures and relatively mild drought stress. Alfredton is near the drier east coast zone, which has harsher summer droughts and rainfall infiltration is limited by slope. According to topography and elevation, New Zealand"s grasslands can be divided into three broad groups: high, hill and flat to rolling country (Valentine and Kemp 2007); Alfredton belongs to the hill country group, and all other four sites are on flat to rolling country.

Manawahe, Atiamuri and Tokoroa are located in the Taupo - Rotorua Volcanic Region and the soils are classified as Tephric Recent, Pumice and Allophanic, respectively (Hewitt 1993). Pastures at these study sites ranged from well-established permanent pasture, to 1-year-old, 3-year-old and 5-year-old forest-to-dairy pasture conversions. The permanent sheep pastures at Alfredton were on steep hill soils, classified as Brown Soils (Hewitt 1993), and data were collected from 36 plots on pre-existing field trials, one testing the effect of the application of different amounts of lime and another studying nutrient runoff. The permanent dairy pastures at Colyton, were on a Pallic soil (Perch-gley argillic pallic, Hewitt 1993) and data were collected from 40 plots on a pre-existing nitrogen and phosphorus fertiliser trial.

5.2.2. Acquisition of pasture reflectance factor spectra and pasture samples

At intervals between August 2006 and September 2007, reflectance factor spectra were acquired from pasture plots, followed by cutting of pasture samples for botanical analysis (Table 5.1). Four seasonally based visits were made to Colyton where 40, 2m x 5m experimental plots excluded from grazing were sampled. Alfredton was visited five times, and pasture was sampled from 36, 0.5m x 1m exclusion cages. Similar but smaller 0.36m x 0.60m exclusion cages were sampled at Atiamuri (two field visits), Manawahe (one field visit) and Tokoroa (one field visit).

Table 5.1. Number of pasture samples acquired per site and corresponding date of collection.

Samples (total number of samples per site)	W Aug 2006	Sp Oct 2006	Sp Nov 2006	Su Feb 2007	A Mar 2007	A Apr 2007	A May 2007	W Jun 2007	Sp Sep 2007
Alfredton (171)	28		36	36			35		36
Atiamuri (28)		19				9			
Colyton (160)		40			40			40	40
Manawahe (36)		36							
Tokoroa (9)		9							

W - winter; Sp - spring; Su - summer; A - autumn

Reflectance factor spectra from the pasture swards (Figure 2.1 on Chapter 2) were acquired *in situ* using an ASD FieldSpec® Pro FR spectroradiometer (ASD Inc., Boulder, CO, USA) under artificial illumination provided by the Canopy Pasture Probe (CAPP) top-grip (Chapter 2). A large white ceramic tile was used as a reflectance standard (Sanches *et al.* 2009, Chapter 3). Ten spectra were acquired from each plot in order for the field of view of the FieldSpec® fibre optic sensor in the CAPP to cover the whole area of the pasture harvested from the plot, or exclusion cage.

After the acquisition of reflectance data, each pasture plot was then clipped and the samples were kept refrigerated until the botanical analysis was processed. At the Colyton site, because of the large size of the plots, a representative pasture sample was collected; at the other sites the entire mass under the cage was clipped to grazeable height. In the laboratory, a sub-sample of each sample was separated into grass, legume and weed then each component was weighed and the corresponding (fresh) botanical percentage calculated. As the objective of this study was the quantification of botanical composition (e.g. grass, legume, and weed proportions), individual grass, legume and weed species in the pastures samples were not separated and identified, but a general observation was made on the dominant species present.

5.2.3. Spectral data pre-processing

Pre-treatment of spectral data is crucial because there are several kinds of noise, such as high-frequency noise associated with the instrument‟s detectors and electronic circuits, and baseline fluctuations affecting the spectra (Ozaki *et al.* 2007). In this study, the pre-processing consisted of applying spectral averaging, smoothing and calculating the first derivative of the spectra. The ten spectra acquired per pasture plot were first averaged using the SpectraProc software (Hueni and Tuohy 2006) to form a single spectrum per plot. Then, using The Unscrambler® 9.7 software (CAMO, Oslo, Norway), the spectral data were smoothed by applying a Savitzky-Golay filter (Savitzky and Golay 1964) with window size of 81 and polynomial order of 4, followed by the calculation of the first derivative of the reflectance (FDR) using the Savitzky-Golay algorithm with window size of 3 and polynomial order of 2. The first derivative is one of the most commonly used mathematical transformations applied to reflectance spectra in an attempt to extract spectral features, minimize background noise and enhance weak signals (Demetriades-Shah *et al.* 1990, Tsai and Philpot 1998).

5.2.4. Data analysis

A principal component analysis (PCA) of the pre-processed first derivative spectral data (all samples) was conducted and the scores of the first two principal components (PC1 and PC2) were plotted against each other (plotted per site and per season). In PCA the original highly correlated variables are transformed into uncorrelated new variables (the PCs). The PCs are ordered in relation of the amount of variation they explain, and the first few components retain most of the variation present in the original variables. PCA highlights the differences and similarities in the data, and it is useful to extract information about patterns in the samples (Jolliffe 2002).

The processed data between 420 and 2400 nm (wavelengths between 350-419 nm and 24001-2500 were discarded because of low signal-to-noise ratio) were then regressed against the botanical composition proportions (grass, legume and weed percentages) using partial least squares regression (PLSR), and the regression model internally tested using full cross-validation (leave-one-out method). PLSR was chosen because there is a large amount of data and the predictor variables are highly correlated (the information in single wavelengths is usually highly correlated with information in other wavelengths). That means there is redundant information, but rather than select a few of the predictor variables, it is better to reduce their number to a few components (latent variables). In PLSR the components are linear combinations of the predictor variables chosen so that they describe as much of the variation in the predictors as possible and also give extra weight for variables that show a high correlation with the response variable (Miller and Miller 2005). Cross-validation is an internal calibration method in which the calibration data are also used as validation data, and is often used when observations are limited and/or separate validation sample sets are not available (Davies 1998). Whenever possible in this study (i.e. when a good number of samples were available) regressions using independent calibration and validation sets and full cross-validation were both performed, but because the results of these two methods were very similar, only the results of cross-validation were shown.

Firstly, PLSR was conducted with the entire botanical and spectral datasets (404, 365 and 350 samples for grass, legume and weed predictions, respectively; samples with no legume and weed components were discarded from the corresponding regressions). Then PLSRs were conducted on data from the two sites that had a good number of samples (> 100): Alfredton (n = 171) and Colyton (n = 160). For these two sites PLSRs were also conducted for each sampling date at Alfredton (Aug-06, Nov-06, Feb-07, May-07 and Sep-07), and Colyton (Oct-06, Mar-07, Jun-07 and Sep-07). For Colyton, PLSRs were also carried out for the samples grouped by treatments: *added P* and *added P+N* samples, which correspond to the plots where P fertiliser and a combination of P and N fertiliser had been applied, respectively. In addition regressions were carried out for samples grouped by season (Spring: Alfredton Nov-06 and Sep-07, Colyton Oct-06 and Sep-07, Atiamuri Oct-06, Manawahe Oct-06, and Tokoroa Oct-06. Winter: Alfredton Aug-06 and Colyton Jun-07. Autumn: Colyton Mar-07, Atiamuri Apr-07 and Alfredton May-07).

The accuracy and precision of the PLSR models were assessed using the following statistics, coefficient of determination (R^2), root mean square error of cross-validation (RMSECV), regression slope, bias and the ratio of standard deviation (SD) of the nutrients measured to the RMSECV (SD/RMSECV). Accurate and precise prediction is shown by high R^2, low RMSECV, slope close to one, bias close to zero, and high SD/RMSECV. The R^2 of validation indicated how well the model predicted responses for unknown observations. The RMSECV provides a direct estimate of the modelling error expressed in original measurements units (percentage of grass, legume and weed in the pasture samples). The regression slope is the amount of change in the dependent variable (pasture botanical composition) that is associated with a change in the independent variable (pasture spectral data). The bias is the systematic difference between predicted and measured values (average value of the residuals). The SD/RMSECV ratio enables the evaluation of the RMSECV in terms of SD of the reference data (pasture nutrients measured), making possible the comparison of the prediction ability of models developed for datasets which have different sample ranges.

5.3. Results and discussion

5.3.1. Pasture botanical components

All samples (404) collected in this study had a grass component, 90% a legume component, and 87% a weed component. With the exception of the Alfredton site, the most common grass species was perennial ryegrass; and legume, white clover. All plant species not belonging to grass or legume families were classified as weeds. These included buttercups (*Ranunculus spp.)*, catsear (*Hypochaeris radicata)*, chickweed (*Stellaria media)*, daisy (*Bellis perennis*), docks (*Rumex spp.*), hydrocotyle (*Hydrocotyle spp.)*, plantain (*Plantago spp.)*, red dead-nettle (*Lamium purpureum)*, thistle (*Cirsium arvense)* and yarrow (*Achillea millefolium)*. The hill country pasture at Alfredton presented the greatest diversity in pasture species, with browntop the dominant grass species.

According to Suckling (1975), an ideal legume proportion in a pasture sward should be at least 20-25%; Kemp *et al.* (1999a) suggested that for sufficient N fixation, clover should be higher (25-35%) in white-clover based pastures. Only in summer was the legume content of the hill pasture (Alfredton) > 20% (Table 5.2). For the lowland sites legume percentages were > 20% in all seasons except winter.

In New Zealand pasture systems the complementary behaviour of summer-autumn growth patterns of legumes and winter-spring dominance by temperate grass growth is exploited (Brock *et al.* 1989). This oscillatory behaviour of grass-legume swards is naturally balanced, the improvement of grass growth occurs via enhanced legume N fixation, followed by legume inhibition by competitive grass growth (Valentine and Matthew 1999). This seasonality in pasture botanical composition was apparent at both Alfredton and Colyton sites (Figure 5.2), where the legume content was highest and grass content lowest in summer and the opposite case occurred in the winter. In summer, the grass growth was limited because the grass enters the reproductive growth phase and moisture stress may also occur; at the same time, white clover has its maximum production because of its higher optimum temperature of growth (Valentine and Kemp 2007).

Table 5.2. Basic statistics: minimum (Min.), maximum (Max.), range, mean and standard deviation (SD) for grass, legume and weed percentages (in fresh weight basis) in each dataset analysed.

Sample set	Grass (%)					Legume (%)					Weed (%)				
(n. of samples)	Min.	Max.	Range	Mean	SD	Min.	Max.	Range	Mean	SD	Min.	Max.	Range	Mean	SD
ALFREDTON															
Aug-06 (28)	84	99	15	92	5	0	11	11	3	3	1	13	12	4	3
Nov-06 (36)	33	93	60	72	15	1	57	56	15	14	1	36	35	13	8
Feb-07 (36)	26	95	69	68	17	2	70	68	21	16	0	31	31	11	8
May-07 (35)	75	99	24	91	6	0	13	13	3	3	0	22	22	6	6
Sep-07 (36)	73	99	26	90	7	0	5	5	1	1	0	24	24	9	7
TOTAL (171)	26	99	73	82	15	0	70	70	9	12	0	36	36	9	7
COLYTON															
Oct-06 (40)	57	100	43	88	11	0	39	39	7	9	0	35	35	5	8
Mar-07 (40)	17	100	83	70	25	0	79	79	21	23	0	25	25	8	7
Jun-07 (40)	70	100	30	91	8	0	22	22	4	6	0	22	22	5	5
Sep-07 (40)	56	100	44	85	12	0	32	32	10	10	0	24	24	5	5
Added P+N (80)	68	100	32	93	7	0	15	15	2	3	0	28	28	5	6
Added P (80)	17	97	80	74	19	0	79	79	20	17	0	35	35	7	7
TOTAL (160)	17	100	83	83	17	0	79	79	11	15	0	35	35	6	7
MANAWAHE															
Oct-06 (36)	44	95	51	65	11	4	33	29	20	8	2	41	39	15	10
ATIAMURI															
Oct-06 (19)	45	99	54	72	17	0	55	55	25	18	0	17	17	3	6
Apr-07 (9)	9	92	83	64	29	5	91	86	32	31	0	15	15	4	5
TOTAL (28)	9	99	90	70	21	0	91	91	27	22	0	17	17	3	5
TOKOROA															
Oct-06 (9)	45	99	54	80	16	0	28	28	12	9	0	27	27	8	10
SEASONS															
Spring (216)	33	100	67	79	15	0	57	57	12	13	0	41	41	8	9
Winter (68)	70	100	30	91	7	0	22	22	4	5	0	22	22	5	4
Autumn (84)	9	100	91	78	23	0	91	91	15	22	0	25	25	7	7
TOTAL (404)	9	100	91	80	17	0	91	91	12	15	0	41	41	8	8

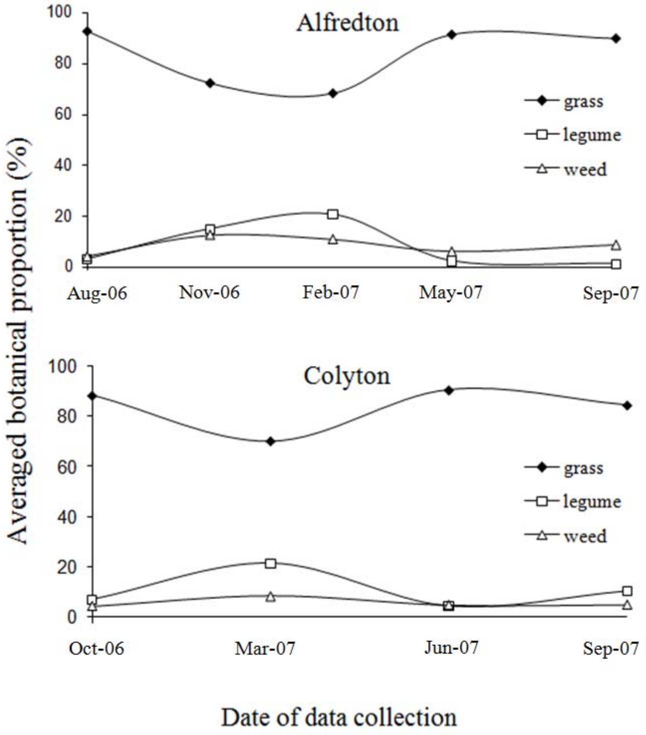

Figure 5.2. Botanical proportions (grass, legume and weed) averaged per date of data collection for Alfredton and Colyton sites.

At Colyton, the averaged percentage of legume in the *added P* plots (which received applications of P fertiliser) was 20%, approximately 10 times higher than that of the *added P+N* plots (which received applications of both P and N). In New Zealand, P fertilisers are applied to maintain or increase pasture production by encouraging legume growth and therefore N fixation. Grasses are more competitive than legumes in taking up available N from the soil and when N fertiliser is applied legume yield and N fixation rate are reduced (Kemp *et al.* 1999a).

Legumes have a major impact on the pastures nutrient value for animals, as do low palatability weeds (Litherland and Lambert 2007). Although some weeds supply nutrients, most common weeds (such as buttercup, docks and thistles) found in New Zealand pastures have low feed quality (Stewart and Charlton 2006), thus the number of weeds should be kept minimal. From the dataset collected the weeds percentage varied from 0% to 41%, with an average of 8% (Table 5.2); and 21% of the samples (73 of 350) contained over 15% of weeds, with the majority of weeds found in the pastures in mid-late spring and summer.

5.3.2. Pasture spectra

There was more variation in the pasture reflectance factor spectra at the Alfredton hill site than the lowland Colyton site, due to the greater diversity in sward attributes including botanical composition, presence of non-green components (seed heads, dead matter, and flowers), pasture height and pasture sward cover at the hill pasture sites (Figure 5.3). For example, the amplitude of the variation of the reflectance factor spectra between 700 nm and 1300 nm was 50% higher among Alfredton spectra than among Colyton spectra (Figure 5.3a). In those wavelengths (near infrared) the spectral response of plants is dominated by leaf internal structure. Different plant species may have distinct leaf internal structure with different distribution of intercellular air spaces and arrangement and size of cells that affect the way the light passes through leaves and consequently the amount of light that is reflected by the leaves (Kumar *et al.* 2001). The amplitude of the variation of the reflectance factor spectra between 700 nm and 1300 nm was also higher among summer spectra (which has higher diversity in sward attributes) than among winter spectra (Figure 5.3b), providing further support to the explanation above.

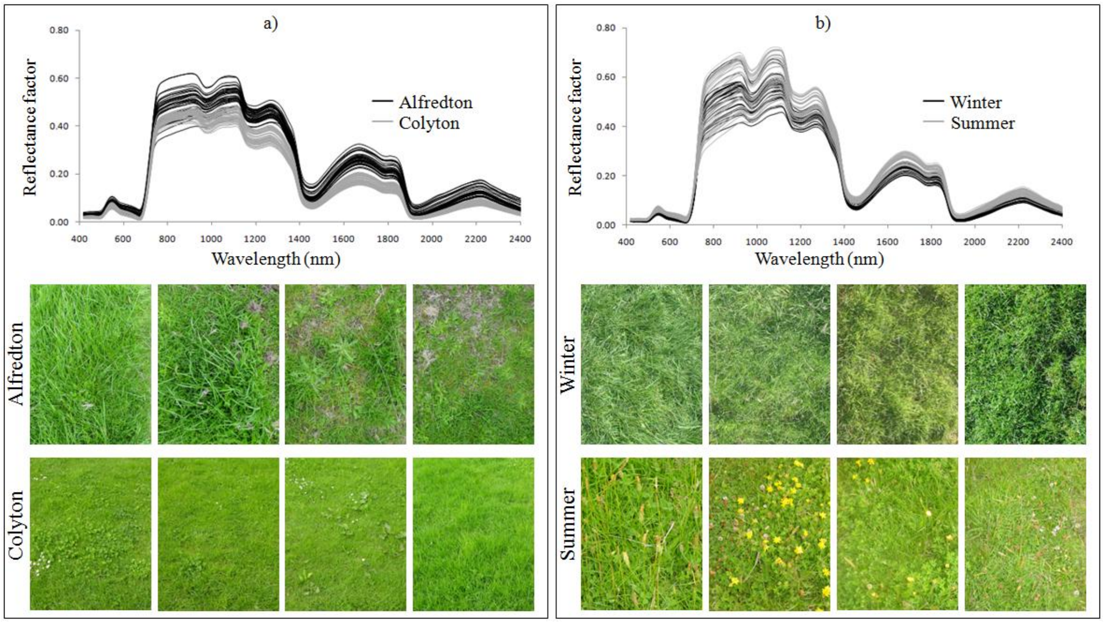

Figure 5.3. Reflectance factor spectra of pasture swards collected in different seasons and sites and photographs illustrating some of the plots measured: a) spectra collected during early spring at Alfredton and Colyton; b) spectra collected at Alfredton during winter and summer.

An efficient way to analyse the distribution of spectral data of various samples is to conduct a PCA. The sum of the variance accounted for in PC1 and PC2 (Figure 5.4) is 85%, so the PCA score plots (PC1, PC2) can be interpreted with a high degree of confidence. Samples closer to each other in the score plots are similar to each other and conversely samples far away from each other are different from each other (Figure 5.4).

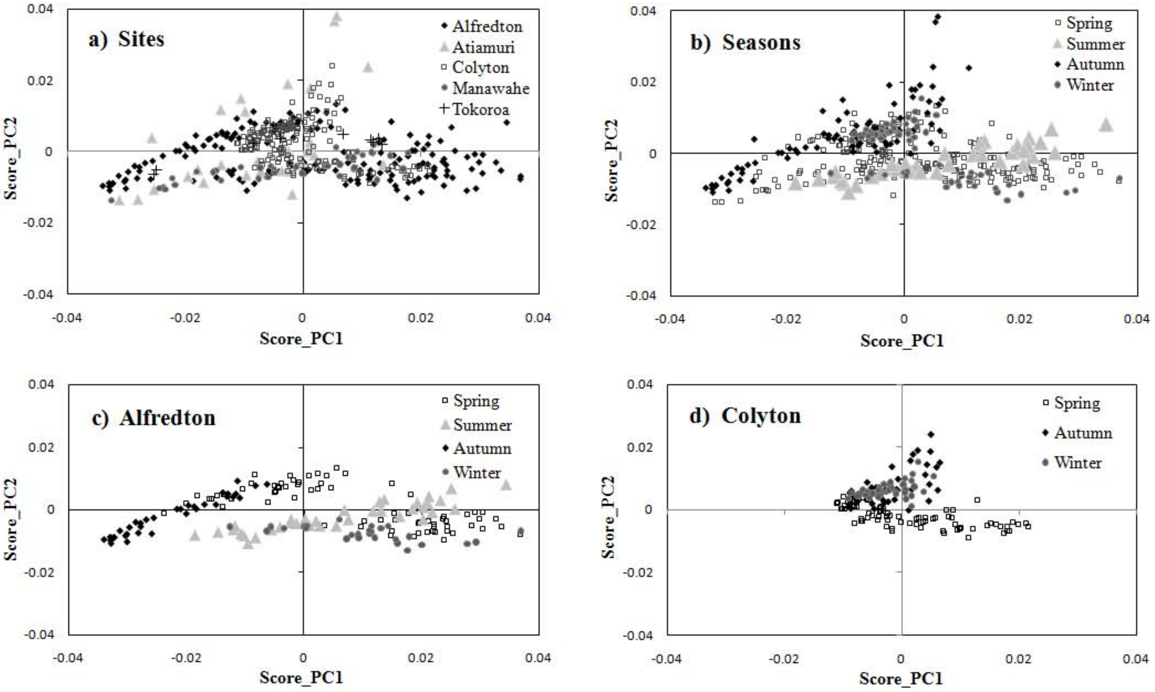

Figure 5.4. PCA score plots for entire FDR dataset (n = 404): a) plotted per site and b) plotted per season. PCA score plots for c) Alfredton samples (n = 171) and d) Colyton samples (n = 160) plotted per season.

It is clear in the PCA score plots that there is more variation in the spectral data collected from the Alfredton site (Figure 5.4c) than from Colyton (Figure 5.4d). Compared to Colyton, which was a high fertility lowland site, the plots analysed at the Alfredton site were located on steep and moderate slopes with north, south and east-facing aspects. The diversity in botanical composition in New Zealand hill pastures is highly influenced not only by rainfall, temperature and soil moisture, but also by altitude, slope, aspect and micro-topography (Nicholas 1999). Normally rainfall increases and temperature decreases with altitude. In addition, different aspects receive differing solar radiation inputs, have different temperatures and effective rainfall (the proportion of runoff increases with increased slope) resulting in differing soil moisture regimes, (McKenzie *et al.* 1999). Accordingly species diversity is much higher in the hill country than in lowland pastoral systems (Matthew *et al.* 1988). Moreover, the Colyton data were collected from a dairy pasture under a cutting (non-grazed) regime compared with the sheep grazed pasture at Alfredton, where the pasture samples were collected from under exclusion cages. Thus the sample variability in the exclusion cage was influenced by the uneven return of sheep dung and urine. Consequently, the data collected from the Alfredton hill pasture in this study reflect not only the greater pasture species diversity, but also the influence of nutrient return by animals, in addition to the effect of greater variation in soil fertility. The PCA score plots (Figure 5.4c and 5.4d) confirm that this variation observed in the field is also observed in the spectral data.

The degree of variability in the spectral data changes with season (Figure 5.4b, PCA score plot). The variability is caused largely by the Alfredton hill site dataset (Figure 5.4c). Across all sites and samplings, grass content in the sward was greater in winter than in spring and autumn, while in spring and summer higher legume contents were observed. These observations are consistent with literature reporting cyclical changes in the botanical composition of New Zealand pasture. In the beginning of the year (summer) pastures contain increased dead matter, changing to mainly leafy and vegetative growth in the middle of the year (winter), and increasing stem and reproductive growth at the end of the year (in late spring and early summer) (Holmes *et al.* 2002). The change from vegetative to reproductive growth from early spring through to early summer is accompanied by a change from a vigorous leafy pasture to one dominated by stem and inflorescences as the plant flowers and sets seeds (Chaves 2003).

5.3.3. Predictions of pasture botanical components

It was not possible to build accurate PLSR calibration or validation models (results not shown) between pasture grass, legume and weed percentages (expressed on a fresh weight basis) and the processed reflectance factor data (1981 wavelengths from 420 nm to 2400 nm at 1 nm spacing). However, when the first derivative of the reflectance (FDR) data was calibrated against the pasture botanical composition data using PLSR (cross validation method), some significant relationships were obtained for predictions of unknown pasture botanical composition. The R^2s for the prediction of grass percentage (Table 5.3 and Figure 5.5) varied from -0.04 to 0.85, and the regression slopes between -0.01 and 0.87. In general as the range of observed % grass in the sward increased the ability of the spectral data to predict that range improved. For example, higher R^2s and regression slopes were obtained for data from the Colyton site in March 2007 (grass range = 83%) than in June 2007 (grass range = 30%). Similarly the % grass in autumn samples across all sites (grass range = 91%) was better predicted than spring (grass range = 67%) and winter (grass range = 30%) samplings across all sites. Samples collected at the Alfredton site from May to September were amongst the sample sets with the lowest range of % grass (≤ 32%), along with Colyton *added P+N* samples and winter samples from all sites combined (Table 5.2), which invariably produced PLSR models with little accuracy of prediction of % grass in the sward (Table 5.3). The R^2 statistic can be misleading when used to assess the accuracy of relationships predicting narrow ranges of observed values. The resultant R^2 might be very low but this does not necessarily means the calibration is poor, and for that reason the regression should not be evaluated solely on the R^2. Overall, the bias was small. The highest bias (0.231) was observed for the regression using all Alfredton samples. The RMSECV, the estimate of the error expected, varied from 5% to 17%. The smallest RMSECV were obtained for the samples collected from late autumn to early spring independent of site. For Alfredton the highest RMSECV values were obtained for the seasons (late spring and summer) with higher weed content. However, a better way to evaluate the RMSE, especially when comparing datasets with different ranges of samples, is to use the SD/RMSECV ratio (Table 5.3). SD/RMSECV values near or under one indicate that the predictions are poor and not reliable, since the predicted error is greater or similar to the standard deviation of the samples analysed. For the grass predictions, the SD/RMSECV ratio ranged from 0.9 to 2.5. All Alfredton regressions were inaccurate. Colyton regressions were reasonable for all but the *added P+N* samples and mid spring season. The regression carried out using all samples (n = 404) was also reasonable, as well as the prediction for the autumn season.

Table 5.3. Statistical summary of partial least squares regression (PLSR) models fitted to predict grass percentage in different datasets.

	Season	N. of samples	Latent Variables	R^2	RMSECV	Slope	Bias	SD/RMSECV
All samples	W Sp Su A	404	9	0.58	11	0.62	-0.008	1.5
Alfredton	W Sp Su A	171	3	0.44	12	0.47	0.231	1.3
Colyton	W Sp A	160	8	0.66	10	0.72	-0.096	1.7
Colyton *added P+N*	W Sp A	80	1	0.07	7	-0.04	0.040	1.0
Colyton *added P*	W Sp A	80	4	0.51	13	0.56	-0.165	1.5
Alfredton Aug-06	W (late)	28	1	0.04	5	0.03	-0.025	1.0
Alfredton Nov-06	Sp (late)	36	3	0.23	14	0.31	-0.162	1.1
Alfredton Feb-07	Su (late)	36	2	0.07	17	0.12	-0.095	1.0
Alfredton May-07	A (late)	35	1	0.02	6	-0.03	-0.022	1.0
Alfredton.Sep-07	Sp (early)	36	1	0.02	8	-0.01	-0.067	0.9
Colyton Oct-06	Sp (mid)	40	1	0.02	11	-0.01	0.011	1.0
Colyton Mar-07	A (early)	40	4	0.85	10	0.86	-0.118	2.5
Colyton Jun-07	W (early)	40	4	0.52	5	0.58	0.137	1.6
Colyton Sep-07	Sp (early)	40	4	0.72	6	0.76	0.074	2.0
Spring	Sp	216	6	0.47	11	0.50	0.031	1.4
Winter	W	68	4	0.13	6	0.22	0.074	1.2
Autumn	A	84	8	0.81	10	0.87	-0.170	2.3

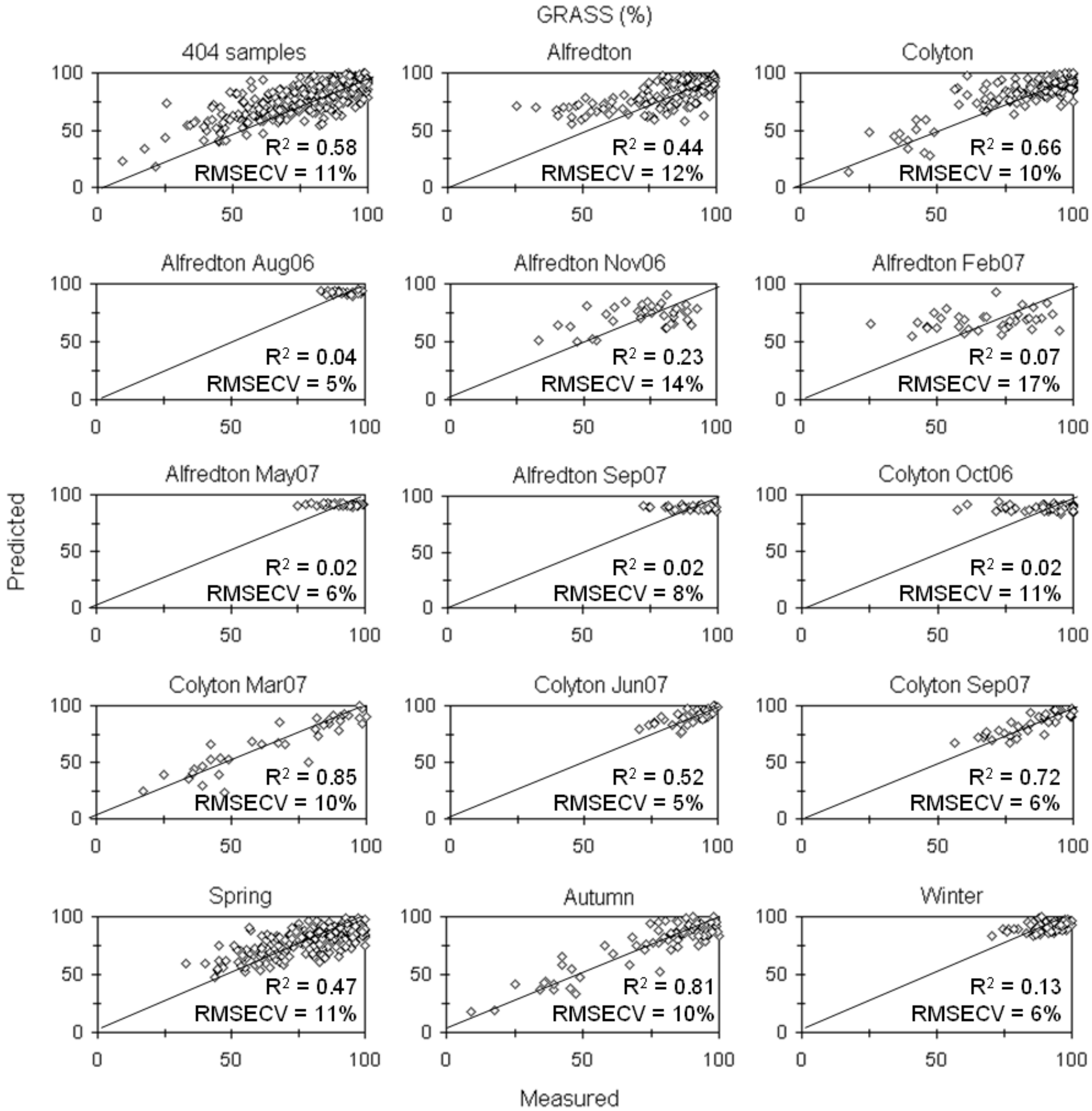

Figure 5.5. Relationships between measured grass percentage (x-axis) and those predicted from the PLSR full cross-validation (y-axis).

The PLSR models developed to predict the % legume component in the sward (Table 5.4 and Figure 5.6) were of similar accuracy to the grass models. The R^2 values for the regression models ranged from 0.02 to 0.80, and the regression slopes between 0.02 and 0.83. The largest R^2 values were again obtained for the Colyton site and for autumn samples. All regressions using sample data exclusively from the Alfredton site and winter samples had very low R^2 values, again mostly associated with observed datasets having small ranges. The RMSECV ranged from 1% to 16%. The smallest errors were regression models built using samples collected from late autumn to early spring, independently of site. For Alfredton the highest RMSECV values were obtained using data from the seasons with higher weed content (late spring and summer). The regressions for Alfredton late spring and Colyton mid spring samples had the highest bias (0.241 and 0.525, accordingly). The SD/RMSECV ratio ranged from 1.0 to 2.2, with the higher values associated with autumn samples (combined sites) and Colyton autumn samples.

Table 5.4. Statistical summary of partial least squares regression (PLSR) models fitted to predict legume percentage in different datasets.

Legume%	Season	N. of samples	Latent Variable	R^2	RMSECV	Slope	Bias	SD/RMSECV
All samples	W Sp Su A	365	9	0.56	10	0.60	0.044	1.5
Alfredton	W Sp Su A	169	4	0.39	10	0.43	0.096	1.2
Colyton	W Sp A	126	10	0.71	9	0.77	0.090	1.7
Colyton *added P+N*	W Sp A	47	1	0.19	3	-0.10	-0.007	1.0
Colyton *added P*	W Sp A	79	4	0.55	11	0.58	0.064	1.5
Alfredton Aug-06	W (late)	28	1	0.07	3	0.06	-0.013	1.0
Alfredton Nov-06	Sp (late)	36	3	0.19	13	0.31	0.241	1.1
Alfredton Feb-07	Su (late)	36	1	0.02	16	0.02	0.117	1.0
Alfredton May-07	A (late)	33	1	0.05	3	-0.04	-0.006	1.0
Alfredton.Sep-07	Sp (early)	36	2	0.05	1	0.10	0.009	1.0
Colyton Oct-06	Sp (mid)	25	3	0.20	8	0.29	0.525	1.1
Colyton Mar-07	A (early)	34	1	0.74	12	0.73	-0.026	1.9
Colyton Jun-07	W (early)	31	3	0.30	5	0.39	-0.113	1.2
Colyton Sep-07	Sp (early)	36	3	0.67	6	0.71	-0.100	1.7
Spring	Sp	194	7	0.46	9	0.52	0.024	1.4
Winter	W	59	2	0.08	4	0.12	0.012	1.3
Autumn	A	76	6	0.80	10	0.83	0.034	2.2

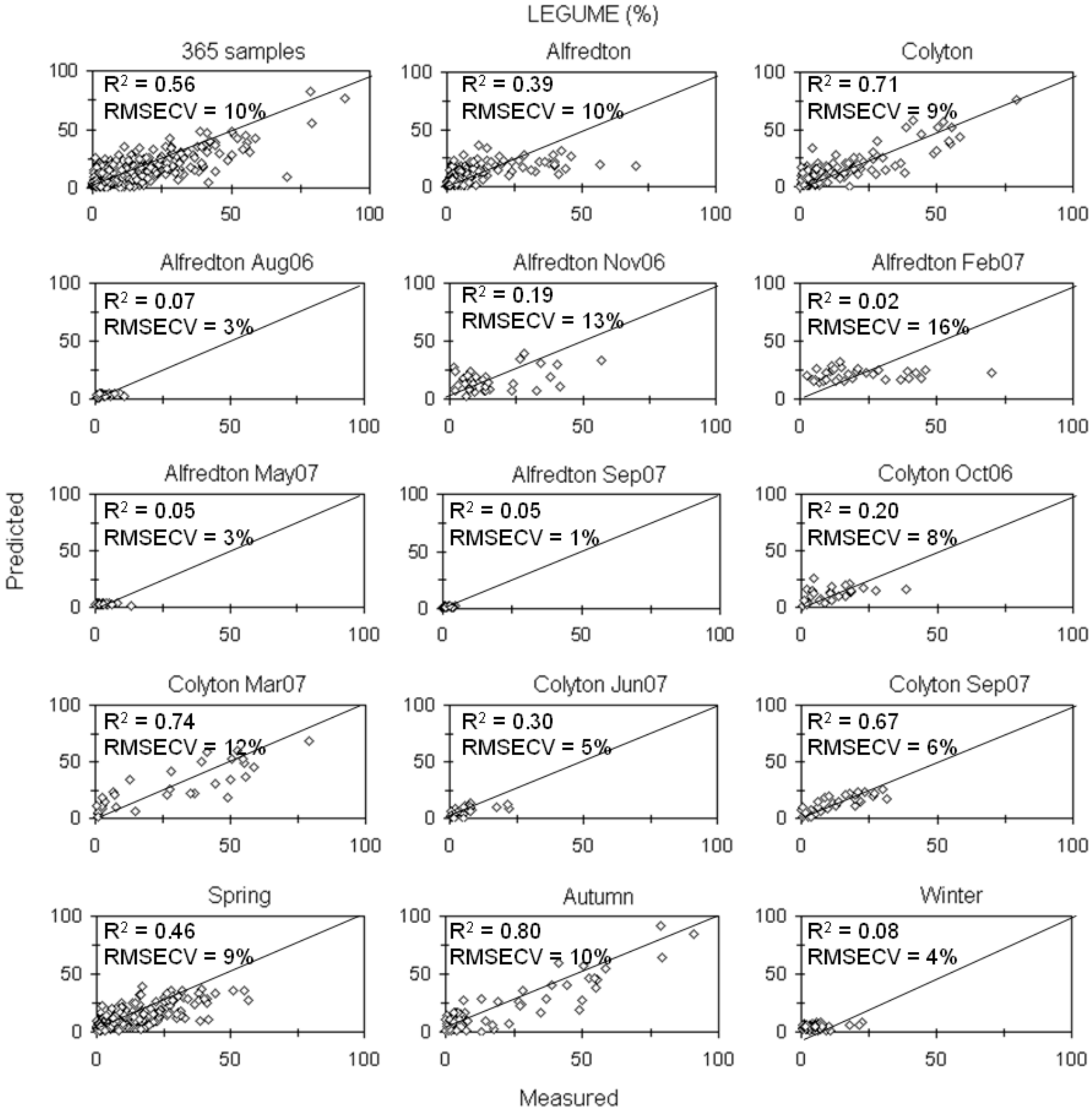

Figure 5.6. Relationships between measured legume percentage (x-axis) and those predicted from the PLSR full cross-validation (y-axis).

The regression models for weed percentage (Table 5.5 and Figure 5.7) had very low R^2 values (0.00 to 0.44) and low regression slopes (≤ 0.21) in all situations. Overall the range of weeds in the samples analysed was very narrow. The RMSECV values ranged between 3% and 12%. The lowest RMSECV (3%) was observed for regression models using samples collected during winter at Alfredton, followed by models using the winter samples of combined sites (4%). The SD/RMSECV statistic (values ≤ 1.1) was low for all weed models and all predictions were inaccurate. The prediction of weed percentage was, in general, the weakest of the three sward components examined. Along with the small absolute percentages and small range of weeds species in the datasets, the poor fit of the regression model might be explained by the fact that several distinct types of plants (buttercup, catsear, chickweed, daisy, dock, hydrocotyle, plantain, red dead-nettle, thistle and yarrow) with different types of leaves and plant structure were classified into one single weed group (weed). In contrast, grass and to a lesser degree the legume components were represented by fewer species and with similar plant structures.

Table 5.5. Statistical summary of partial least squares regression (PLSR) models fitted to predict weed percentage in different datasets.

Weed%	Season	N. of samples	Latent Variable	R^2	RMSECV	Slope	Bias	SD/RMSECV
All samples	W Sp Su A	350	5	0.15	7	0.18	0.009	1.1
Alfredton	W Sp Su A	167	4	0.11	7	0.14	0.019	1.0
Colyton	W Sp A	128	4	0.08	6	0.17	0.017	1.2
Colyton *added P+N*	W Sp A	61	6	0.18	7	0.38	-0.176	0.9
Colyton *added P*	W Sp A	67	3	0.05	7	0.12	0.093	1.0
Alfredton Aug-06	W (late)	28	1	0.03	3	-0.04	0.034	1.0
Alfredton Nov-06	Sp (late)	36	1	0.00	8	0.00	0.025	1.0
Alfredton Feb-07	Su (late)	34	1	0.03	8	-0.03	-0.008	1.0
Alfredton May-07	A (late)	34	1	0.06	7	-0.06	-0.045	0.9
Alfredton.Sep-07	Sp (early)	35	1	0.33	8	-0.13	-0.057	0.9
Colyton Oct-06	Sp (mid)	18	1	0.44	12	-0.20	-0.186	0.7
Colyton Mar-07	A (early)	36	1	0.01	7	0.02	0.036	1.0
Colyton Jun-07	W (early)	36	1	0.09	5	0.12	-0.051	1.0
Colyton Sep-07	Sp (early)	38	4	0.18	5	0.33	-0.004	1.0
Spring	Sp	176	3	0.20	8	0.21	0.001	1.1
Winter	W	64	1	0.02	4	-0.01	0.006	1.0
Autumn	A	76	1	0.02	7	0.03	-0.003	1.0

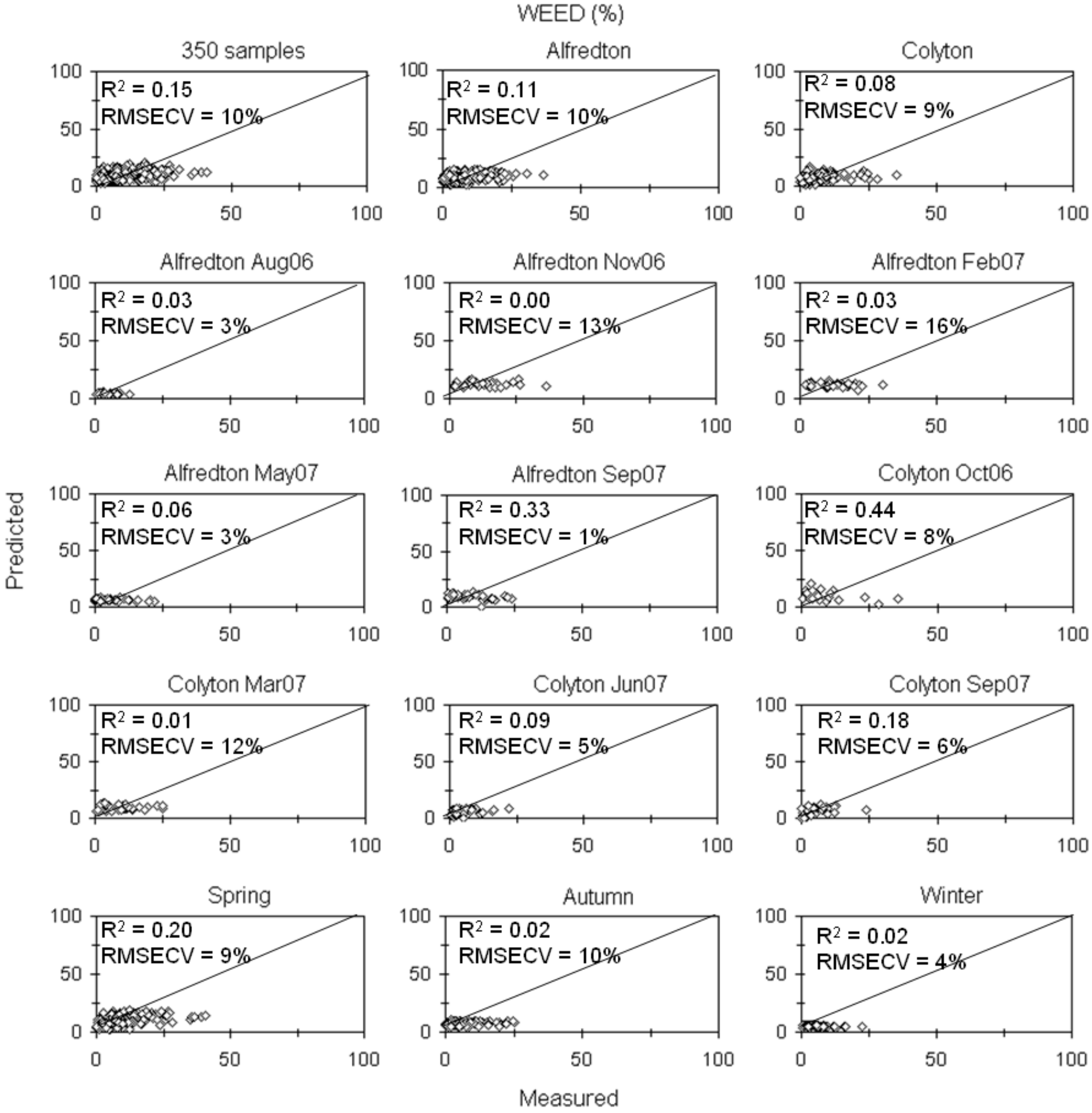

Figure 5.7. Relationships between measured weed percentage (x-axis) and those predicted from the PLSR full cross-validation (y-axis).

5.3.4. PLSR carried out separately for Colyton samples with and without added N

The *added P* and *added P+N* plots at the Colyton site had marked differences in legume and grass percentages. This created a significant range in botanical composition (Table 5.2), that resulted in PLSR calibration models that gave a reasonably accurate prediction of grass and legume contents (Tables 5.3 and 5.4). When the data for the *added P* and *added P+N* plots were separated into individual sample sets (Table 5.3 and 5.4), the range in the grass and legume botanical composition data was less for the *added P+N* set, and the PLSR calibrations for this set were less accurate.

5.3.5. Contrasting predictions for hill (Alfredton) and lowland (Colyton) sites

Despite a similar range of botanical compositions (grass, legume and weed) in samples at the Alfredton (hill country) and Colyton (flat) sites (Table 5.2), the variation of botanical components explained by the Alfredton PLSR calibration models (low R^2s values) was low. The Alfredton pasture sample set includes a greater diversity of grass and legume species compared to the Colyton sample set, which could explain the more complex spectral data and the inaccuracy of the calibration models. The problem is not the high diversity per se, but that this diversity was not taken into account in the botanical separation. In contrast (as described in the previous section), the variation in spectral data at the Colyton site is a direct consequence of the variation in grass and legume components.

When examining the RMSECV, which measures the variability of the difference between predicted and reference values, the results were comparable for both Alfredton and Colyton sites. Since no summer data were available for Colyton, and to make the comparison equal (based on seasons represented) for the entire dataset per site, the summer data (Feb-07) were omitted from the Alfredton dataset and new PLSRs were carried out for grass (135 samples: LV = 4, R^2 = 0.41, RMSECV = 10, slope = 0.44, bias = 0.127, SD/RMSECV = 1.3) and legume (133 samples: LV = 10, R^2 = 0.47, RMSECV = 7, slope = 0.59, bias = -0.048, SD/RMSECV = 1.3). The RMSECV values were indeed similar for both sites (c.f. Tables 5.3 and 5.4), which means the error of prediction (expressed in original measurements units) was similar for both Alfredton and Colyton (hill country and lowland sites, respectively). However, based on the SD/RMSECV ratio, better results were still obtained for Colyton predictions (SD/RMSECV = 1.7 for both grass and legume components).

5.3.6. Contrasting prediction for different seasons

The ability to predict pasture botanical composition from spectral data also varied between seasons. Data for the four seasons were available for the Alfredton site, and although all predictions for this site were inaccurate, useful information could be extracted. Low R^2 values were observed for all seasons (Tables 5.3 and 5.4), and that might be related to the narrow range of grass and legume percentage for winter, autumn and early spring samples (Table 5.2), however that does not explain the inaccurate calibration results for late spring and summer samples. At Colyton, data were available for early and mid spring, autumn and winter seasons. Colyton winter PLSR calibrations were affected by the narrow sample data range, and although the sample ranges were similar in mid and early spring datasets less accurate calibrations were obtained for mid spring samples. Comparing PLSR models across seasons for all sites, autumn presented the more accurate calibration models (also the best range of grass and legume percentage), winter and spring calibration models were less accurate, with winter calibrations highly affected by the narrow range of sample data (Tables 5.3 and 5.4). Confirmation that the winter PLSR models inaccuracy was caused by the narrow range of samples was obtained by conducting new PLSRs using the winter samples and autumn samples combined. The resulting calibration models for grass (152 samples: LV = 7, $R^2 = 0.75$, RMSECV = 9, slope = 0.79, bias = -0.059, SD/RMSECV = 2.0) and for legume (135 samples: LV = 7, $R^2 = 0.79$, RMSECV = 8, slope = 0.80, bias = 0.069, SD/RMSECV = 2.2) were as good as the autumn ones for legume prediction and a little inferior for grass prediction.

The non-green components of a pasture are poor quality components. Good grazing management with regular and significant defoliations focuses on improving feed quality by minimizing reproductive growth (flowering) during spring and the accumulation of dead matter in summer and autumn (Stewart and Charlton 2006). Grazing management varied at each site. At Alfredton, in the periods (mid late spring till autumn) when the pasture had higher non-green components (such as flowers, seed heads, stems and dead matter), the PLSR calibration models inaccurately predicted pasture grass and legume composition. The worse predictions of pasture grass and legume proportions using Colyton samples were obtained with the PLSR calibration models using samples collected during mid spring;

however, in the Colyton site the variation in non-green components was not very perceptible throughout the seasons. In this study the pasture samples were separated into grass, legume and weed components only and senescing leaves were combined with green leaves and flowers. Green plant materials are spectrally different from non-green plant materials. This divergence appeared in the spectral data analysed but was not taken into account in the botanical composition data. Therefore the accurate prediction of the botanical composition of pastures that have few or no senescing component or flowers (winter or pastures with good grazing management such as at Colyton site) is less challenged.

5.4. Conclusions

Reasonably accurate PLSR calibration models can be achieved for predicting pasture grass and legume proportions from proximal sensed sward reflectance. Similar accuracy was not achieved in predicting pasture weed content. Reasonable level of accuracy of prediction could not be achieved with hill pastures (Alfredton), which include a greater diversity of grass and legume species compared to a lowland sample set (Colyton). The most accurate PLSR calibration models for predicting grass and legume composition were achieved in autumn, a period of the year when a wide range of samples could be gathered. In winter the PLSR calibration models were less accurate probably confounded by the extremely narrow ranges of sample values. The low level of model accuracy was overcome by combining winter samples with autumn samples in the regressions. At the hill country site in summer (data only available for the hill country site) it was noticed that even with a good range of grass and legume percentage in the samples (the greatest among all seasons) the PLSR calibration models were very inaccurate. This is probably related to the high proportion of non-green components in the pasture that unfortunately was not quantified in this study.

In this study, whereas there is variability in botanical composition and in spectral data, the two sets of data are not highly correlated as shown by the low coefficients of determination for most sites and seasons. This study separated the pastures samples into grass, legume and weed components, without discriminating if the materials were green or dead matter, vegetative or reproductive. The physiological changes in the pasture sward species with season are revealed in the spectra (colour, seed head and structural change) but not in the botanical separation. If the non-green pasture components had been quantified for each sample, botanical prediction may have been improved for mid spring until summer periods. This is a relevant consideration that would be interesting to be examined in further research.

The limited utility of the technology may be in part a product of the way in which it is applied and evaluated. For example the interest in the identification of weed species in mixed pasture might be in early detection of a potential infestation, rather than the actual percentage. Exploring proximal sensing for early detection before the weed is visually perceptible might be where the technology adds value to pasture management decision making. Similarly with sensing the legume content of the pasture, the interest may only be in establishing if there is sufficient legume (>20%) for finishing young stock and so the level of accuracy achieved here may be adequate.

CHAPTER 6:

Seasonal predictions of *in situ* pasture macro-nutrients in New Zealand pastoral systems using hyperspectral data

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Some results of this study have been presented as a poster in the 13th Australasian Remote Sensing and Photogrammetry Conference, Canberra, Australia, 20-24 November 2006 (abstract); and have been orally presented and published (full paper) in the proceedings of the 14th Australasian Remote Sensing and Photogrammetry Conference, Darwin, Australia, 29-03 October 2008.

Abstract

For optimum nutrient management of grazed pastures, soil and pasture nutrient levels need to be regularly monitored. The development of new approaches for gathering this kind of information can be justified by the twin goals of both economic and environmental sustainability. Remote sensing technologies have been proven very useful for estimating plant biochemistry attributes in laboratory conditions. The challenge is to extend this technology for *in situ* conditions. To evaluate the ability of proximal sensing for predicting pasture macro-nutrients, spectral reflectance measurements between 350-2500 nm were taken from a number of dairy and sheep pastures in New Zealand, in each of the four seasons of the year. Mathematical transformations (absorbance, derivatives, continuum removal) were applied to the reflectance factor spectra and the data regressed against pasture nitrogen (N), phosphorus (P) and potassium (K) concentrations using partial least squares regression (PLSR). Overall more accurate predictions were achieved using the first derivative data. The accuracy of the PLSR calibration models to predict pasture N, P and K concentrations increased with the separation of the pasture samples by season. Predictions with reasonable accuracy ($R^2 > 0.74$ and SD/RMSEP ≥ 2.0) were obtained for N during winter, autumn and summer seasons; P during autumn; and K during summer.

Keywords: nitrogen, phosphorus, potassium, pasture canopy, field spectra, hyperspectral remote sensing.

6.1. Introduction

The productivity of pastoral systems depends to a large extent on soil fertility. In temperate regions, the deficiency of one or more essential mineral nutrients is the most common limitation to optimum pasture growth (McKenzie *et al.* 1999). Although the levels of all macro and micro-nutrients need to be regularly monitored, soil fertility in New Zealand is usually limited by nitrogen (N) and phosphorus (P) availability (Roberts and Morton 1999). In this country, soils are naturally acid and generally deficient in N and P, but other mineral deficiencies can occur, such as potassium (K) deficiency, particularly in intensive dairying systems and pastures cut for silage or hay.

New Zealand pastures are based on a mix of perennial grasses and legumes; with perennial ryegrass (*Lolium perenne*) and white clover (*Trifolium repens*) the most frequently sown species because of their high tolerance to frequent defoliation and treading by livestock. The grass-legume mix is a successful combination because the grass provides the majority of herbage, while the legume species supply symbiotically fixed N and has a high nutritive value (Kemp *et al.* 1999b). The establishment and maintenance of perennial ryegrass and white clover based pastures in New Zealand requires regular input of P and S, while the major N input is obtained through biological fixation of atmospheric N (N_2) by legumes. The primary source of K is acquired mostly by mineral weathering in most soils (During 1972).

Nutrient availability not only dictates pasture growth rates but also influences botanical composition and the phenology of the pasture species. Nitrogen is required by plants for synthesis of chlorophyll and proteins (Moot *et al.* 2007). From 40 to >200 kg ha^{-1} year^{-1} of N can be fixed by legume-based pastures in New Zealand (Hoglund *et al.* 1979). Most of fixed N is cycled within the grazing system. In grazing systems N is lost in harvesting of animal products (20-60 200 kg ha^{-1} year^{-1}) but more significantly it is lost through non-uniform transfer to small areas of the grazed pasture (e.g. stock camps) and from urine patches through both gaseous and leaching losses (Ledgard 2001). Most New

Zealand pastures are N deficient because of the dependence on legume growth, its seasonality and the inefficient transfer of N to the grass species (White and Hodgson 1999). In high fertility lowland pastures with optimum soil test values, 25-30% legume in the sward is ideal for adequate N fixation (Moot *et al.* 2007). Often the legume content is much lower. In hill country the legume component can be very low, particularly in undeveloped pastures (Lambert *et al.* 1986) and it is common for hill pasture growth to be very N limited.

Phosphorus is another key nutrient for plants; it is required in photosynthesis and is found in plant membranes and in the DNA. Phosphorus deficiency can cause early senescence of old leaves and stunting of young leaves. In New Zealand pastoral systems, P is also important for the maintenance of the legume component in the pastures (During 1972). Response of pasture to P application is explained by legume growth being stimulated which in return fixes more N. This N simulates better grass growth. Phosphorus availability for uptake by plant roots is controlled by the rate of P adsorption, fixation and immobilisation in soil. Removal of P from the plant available pool by these processes is ongoing and is the main reason why P fertiliser topdressings are required annually. Other reasons are that P losses can occur in surface runoff, erosion and P removal by animal products (McDowell *et al.* 2005).

Potassium (K) is essential for plants to maintain the electrical balance of cell membranes, for enzyme activation, carbohydrate production and transport and stomatal activity (Lanyon and Smith 1985). Losses of K in grazed pastures result from K removal in animal products, removal of herbage K in hay and silage crops, transfer of excreta to unproductive areas of the farm (e.g. shed, raceways) and K leaching (Williams 1988). Potassium is also necessary to maintain the legume in the grass-legume based pastures; low exchangeable K concentrations in the soil can cause poor legume establishment since grasses are more competitive than legumes in extracting K from the soil (Roberts and Morton 1999). Pasture yield might be reduced if soil K levels are below the critical concentration; on the other hand, the presence of high levels of K can decrease the plant uptake of ions such as Ca^{++}, Mg^{++} and Na^{+} (Barber 1984).

Optimum nutrient management of grazed pastures requires knowledge of both soil and pasture fertility. This can only be achieved by regular monitoring. In grazed pastures monitoring is made more difficult as the animal, in the process of foraging, excretes highly concentrated minerals in patches. Excreta returns are also strongly influenced by the biophysical features of the landscape, with distinct stock camps receiving disproportionate amounts of excreta, while other areas receive little or no excreta (Gillingham 1980). The net effect is high spatial variance in soil nutrient levels and corresponding pasture nutrient concentrations. Approaches capable of measuring and reporting on nutrient content of pasture across the whole farmed landscape, such as with the use of remote sensing techniques, offers the option of differential management of the landscape. Traditional pasture sampling is generally limited to bulking of samples from along fixed transects in just one or two paddocks of a farm, limiting the scope for nutrient management at the paddock scale. While the cost of differential fertiliser application often outweighs the agronomic benefits, limiting excessive nutrient application to landscape units has other advantages. This could include limiting excessive pasture N content as it leads to high N in animal urine, through to high soil P levels contributing to surface water contamination.

Near infrared reflectance spectroscopy (NIRS) has been used to successfully predict foliar biochemical concentrations in dried ground forage leaves under controlled laboratory conditions (Roberts *et al.* 2004). NIRS made it possible to estimate forage quality composition more quickly and cheaply than by wet chemistry methods (Berardo 1997 and Corson *et al.* 1999). New methods using laboratory spectroscopy were developed for estimating the biochemistry of dried leaves, such as the use of continuum-removed analysis by Kokaly and Clark (1999) which was further explored by Curran *et al.* (2001) using a wider range of wavelengths and by Mutanga *et al.* (2005) for measuring pasture canopy parameters. The challenge is to apply remote sensing techniques for the estimation of plant biochemical attributes directly in the field. This would save time, labour, and costs related to the collection, transportation and preparation of samples, and more importantly allow the "mapping" of the plant nutrient profile across the landscape.

Several factors need to be taken into account when extending reflectance measurements from dried ground leaves to fresh canopies (Huang *et al.* 2004). These include absorption bands in the infrared wavelengths being masked by leaf water, variation in leaf internal structure, atmospheric and background effects (Yoder and Pettigrew-Crosby 1995). While some research has been conducted in this area, and promising results were obtained in prediction of pasture parameters *in situ* (Mutanga *et al.* 2004, Sanches *et al.*

2006, Thulin *et al.* 2006, Kawamura *et al.* 2006), testing and improvements of in field techniques are still required.

In this paper the ability to predict the N, P and K concentration of pasture in different seasons of the year under mowing and sheep and cattle grazing using *in situ* hyperspectral remote sensing data, was examined.

6.2. Material and methods

6.2.1. Study sites

The study areas were located on seven farms in the Waikato (Hamilton), Taupo-Rotorua (Tokoroa, Atiamuri and Manawahe), Wairarapa (Alfredton and Woodville) and Manawatu (Colyton) regions, of the North Island of New Zealand (Figure 6.1).

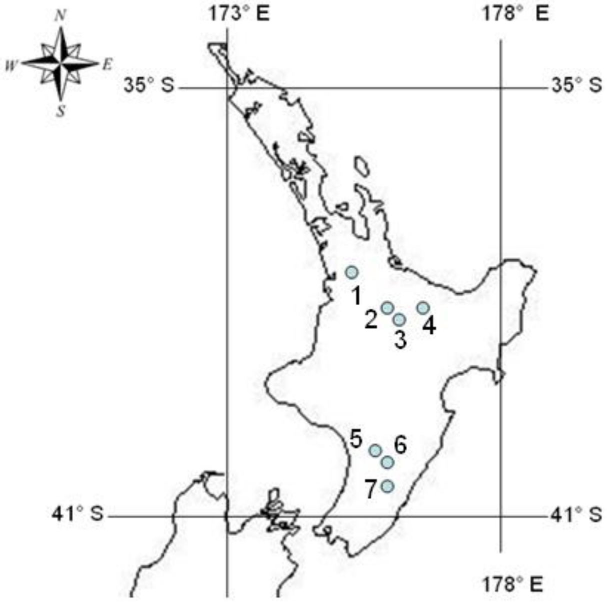

Figure 6.1. Sampling site locations: 1) Hamilton – Ruakura, 2) Tokoroa, 3) Atiamuri, 4) Manawahe, 5) Colyton, 6) Woodville – Ballantrae, and 7) Alfredton.

At Alfredton paddocks covering different aspects (North, South and East) and slopes (gentle and steep) under sheep grazing were selected. At Woodville on the AgResearch "Ballantrae" Hill Country Research Station, one low-fertility and one high-fertility site under sheep grazing were selected. At Colyton, and at Hamilton (on the AgResearch "Ruakura" Research Station), 40 plots, on irrigated dairy pastures within experimental areas receiving different amounts of P and N and cut by mower (instead of grazed), were sampled. At Tokoroa, Atiamuri and Manawahe, pastures ranging from well-established permanent pasture, to 1-year-old, 3-year-old and 5-year-old forest-to-dairy pasture conversions under dairy cow grazing were sampled.

6.2.2. Field data collection

Data were collected from August 2006 to September 2007 in the four seasons of the year (Table 6.1), with spring being defined as September to November; summer as December to February; autumn as March to May; and winter as June to August. Reflectance factors of the pasture swards were acquired *in situ* using an ASD FieldSpec® Pro FR spectroradiometer (ASD Inc., Boulder, CO, USA) under artificial illumination provided by the Canopy Pasture Probe (CAPP) top-grip (Chapter 2). The area measured by the CAPP corresponds closely to a circle with a diameter of approximately 20 cm. The reflectance standard was a white ceramic tile described by Sanches *et al.* 2009 (Chapter 3). At each plot, 10 reflectance factor spectra were acquired and subsequently averaged to form a single reflectance factor spectrum per plot. After the acquisition of spectral data, each plot was then clipped to grazeable height and the pasture samples were sent to be analysed for N (Kjeldahl digest and analysis colorimetrically by FIA with a modified Berthelot reaction) and P and K (Nitric-Perchloric digest and analysis by ICP-OES) using wet chemistry.

Table 6.1. Number of pasture samples collected per site and per season which were analysed for N (n = 357), P (n = 474) and K (n = 377) concentration.

Site /	Winter 06	Spring 06			Summer 06-07			Autumn 07			Winter 07			Spring 07
Season	Aug	Sep	Oct	Nov	Dec	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep
N%														
Alf	12	-	-	12	-	-	12	-	-	12	-	-	-	-
Bal	-	-	30	-	-	-	30	-	-	-	-	-	-	-
Col	-	-	40	-	-	-	-	40	-	-	40	-	-	40
Man	-	-	30	-	-	-	-	-	-	-	-	-	-	-
Rua	-	-	-	-	-	40	-	-	-	-	-	-	-	-
Tok	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Atia	-	-	10	-	-	-	-	-	9	-	-	-	-	-
P%														
Alf	34	-	-	36	-	-	35	-	-	36	-	-	-	-
Bal	-	-	30	-	-	-	30	-	-	-	-	-	-	-
Col	-	-	40	-	-	-	-	40	-	-	40	-	-	40
Man	-	-	36	-	-	-	-	-	-	-	-	-	-	-
Rua	-	-	-	-	-	40	-	-	-	-	-	-	-	-
Tok	-	-	9	-	-	-	-	-	-	-	-	-	-	-
Atia	-	-	19	-	-	-	-	-	9	-	-	-	-	-
K%														
Alf	22	-	-	24	-	-	23	-	-	24	-	-	-	-
Bal	-	-	30	-	-	-	30	-	-	-	-	-	-	-
Col	-	-	40	-	-	-	-	40	-	-	40	-	-	40
Man	-	-	6	-	-	-	-	-	-	-	-	-	-	-
Rua	-	-	-	-	-	40	-	-	-	-	-	-	-	-
Tok	-	-	9	-	-	-	-	-	-	-	-	-	-	-
Atia	-	-	9	-	-	-	-	-	-	-	-	-	-	-

Alf – Alfredton; Bal – Ballantrae; Col – Colyton; Man – Manwahe; Rua – Ruakura; Tok – Tokoroa; Ati - Atiamuri

6.2.3. Spectral data pre-processing

Pre-processing the raw spectral data in this study consisted of averaging, de-stepping, smoothing, removing wavelengths with high signal-to-noise ratio (SNR), and removing bad spectra. The ten spectra acquired per pasture plot were first averaged using the SpectraProc software (Hueni and Tuohy 2006) to form a single spectrum per plot. After that, a de-step procedure using ENVI 4.3 plus IDL 6.3 (Daniel, P., Ticehurst, C., and Thulin, S., personal communication, 29 March 2007) was applied to correct for „steps‟ observed at 1000 and 1800 nm, the splice points between the three detectors - VNIR, SWIR1 and SWIR2 - in the FieldSpec® Pro FR (ASD 2000). Field spectra containing steps are common in instruments that use multiple detectors and to discover the source of steps can be very difficult (Milton *et al.* 2007). The main contributors to „steps" are non-uniform target geometry; different spots on the target are illuminated with different energy density levels; and each fibre in the bundle of the bare input cable sees a slightly different field due to slightly offset fibre fields-of-view, so the actual field-of-view seen by the three spectrometers is different (MacArthur 2007).

Using The Unscrambler® 9.7 software (CAMO, Oslo, Norway), the spectral data were smoothed by applying a Savitzky-Golay filter using 81 smoothing points and polynomial order 4. The selection of the smoothing points and polynomial order were based on experience with previous analysis (Sanches *et al.* 2008). After the smoothing filter had been applied, the spectral data between 390-419 nm and over 2400 nm still presented considerable noise, and for that reason those wavelengths were discarded. The spectrum of one of the 474 pasture samples collected was markedly different from all others (did not presented the typical green vegetation curve) and was discarded from the dataset to be analysed. The final pre-processed data included 473 samples with reflectance factor measured between 420-2400 nm.

6.2.4. Transformations

Three mathematical transformations - absorbance, derivative and continuum removal - were applied to the pre-processed reflectance factor data to test if the transformations could improve the predictions. In spectroscopy analysis absorbance is frequently used because of the near linear relationship with the concentration of an absorbing chemical. Derivative methods have also long been used in spectroscopy studies for resolution enhancement and baseline correction purposes. Tsai and Philpot (1998) indicated such analysis can be particularly effective when analysing hyperspectral data. The continuum removal method, initially used in geological remote sensing (Clark and Roush 1984), was first applied to vegetation studies by Kokaly and Clark (1999), who used the band-depth analysis of absorption features to determine dry leaf biochemistry. Sensitivity to common influences of soil background and atmospheric absorption is reduced by the normalized band depth approach. More recently Mutanga *et al.* (2004) further explored the continuum removal technique by proposing and testing two new variables: normalized band depth index (NBDI) and continuum-removed derivative reflectance (CRDR) to estimate *in situ* pasture quality.

The final forms of spectral data which were analysed in this study were:

- Reflectance factor (R);
- Absorbance (Log(1/R));
- First derivative reflectance (FDR): Calculated by applying the Savitzky-Golay derivative on the reflectance factor spectra;
- First derivative absorbance (FDLog(1/R): Calculated by applying the Savitzky-Golay derivative on the absorbance spectra;
- Continuum removed reflectance (CR): The continuum removed spectra is calculated by dividing the original reflectance factor value for each waveband in the absorption feature by the reflectance factor level of the continuum line (convex hull) at the corresponding wavelength (Kokaly and Clark 1999). In this study the continuum removal was applied to the absorption features between 420-518 nm, 550-750 nm, 910-1081 nm, 1116-1284 nm, 1720-1786 nm, 2010-2196 nm and 2222-2378 nm. These absorption features were selected based on previous research (Kokaly and Clark 1999, Curran *et al.* 2001, Mutanga *et al.* 2004, Huang *et al.* 2004);
- Continuum-removed derivative reflectance (CRDR): Calculated by applying the Savitzky-Golay derivative on the continuum removed reflectance;
- Band depth ratio (BDR): Calculated by dividing the band depth (absorption band depth relative to the continuum) of each waveband by the band depth at the band centre (maximum band depth); and
- Normalized band depth index (NBDI): Calculated by subtracting the band depth from the band centre and dividing it by their sum.

6.2.5. Regression analyses

6.2.5.1. Entire dataset (357 samples for N%, 473 samples for P% and 376 samples for K% prediction)

The difference in dataset size used for calibration of N, P and K concentration against spectral data is a consequence of the different experimental sources of data used in the study. Data were collected from a number of experiments established for other purposes. The K concentration was not obtained in all these experiments. Although in all experiments N and P concentration of the samples were obtained, N concentration of some samples was analysed using NIRS, while others were analysed using wet chemistry methods. In this study only the samples analysed by wet chemistry were used.

The original reflectance factor dataset (R) and the mathematically transformed datasets (Log(1/R), FDR, FDLog(1/R), CR, CRDR, BDR, NBDI) were regressed against pasture N, P and K concentration (%) using partial least square regressions (PLSR) in The Unscrambler® 9.7 software. The number of latent variables (LV) (also known as principal components - PCs) used for each PLSR model represents the number at which additive prediction errors are minimized. The samples were split into calibration and validation datasets (N: 179 calibration and 178 validation samples; P: 237 calibration and 236 validation samples; K: 188 calibration and 188 validation samples). The allocation of data to calibration and validation sets was conducted using prior knowledge of the range of the samples from the chemical analyses (Kusumo *et al.* 2008). The samples were ranked from the lowest to the highest nutrient concentration (N, P and K, separately) and odd and even ranked samples were allocated for calibration and validation sets, correspondingly.

The accuracy and precision of the PLSR calibration models were assessed in terms of coefficient of determination (R^2), root mean square error of prediction (RMSEP), slope, bias and the ratio of standard deviation of the nutrients measured to the RMSEP (SD/RMSEP) for the validation dataset. Accurate and precise prediction is shown by high R^2, low RMSECV, slope close to one, bias close to zero, and high SD/RMSECV.

The SD/RMSEP ratio (or SD/RMSECV, for cross-validation) (previously used by Chang *et al.* 2001 and Thulin *et al.* 2006) is a modification of the ratio of prediction to standard deviation (RPD) developed for NIRS analysis (of dried samples) in agricultural commodity applications, which is calculated by dividing the SD of the measured data in the validation dataset by the standard error of prediction (SEP) corrected for bias (Williams

2001). In Williams (2001), for a regression model to be considered acceptable it needs to have a RPD value higher than 3. However, some studies have reported that a model with a SD/RMSEP or RPD ≥ 2 would provide an excellent calibration model (Chang *et al.* 2001, Dunn *et al.* 2002 and Cohen *et al.* 2005), since the measured variability of the difference between the predicted and measured values for a set of validation samples will be half (or less) of the SD of the measured data.

6.2.5.2. Seasonal datasets

The pasture samples and respective spectra were also grouped according to season. For the samples analysed for N% the seasonal dataset consisted of 162 spring, 82 summer, 61 autumn and 52 winter samples. For P%, 210 spring samples were analysed, 105 summer, 84 autumn and 74 winter samples. For K% there were 158 spring, 93 summer, 63 autumn and 62 winter samples. The data for each season were regressed against pasture N, P and K concentration using PLSR. Because some datasets did not have sufficient samples to be divided into calibration and validation subsets, all the regressions carried out per season used full cross-validation (leave-one-out) as the validation method. Preferably, according to Malley and Martin (2003), a calibration subset should have a minimum of 100-150 samples.

The accuracy of the PLSR models carried out per season was assessed in terms of coefficient of determination (R^2), root mean square error of cross-validation (RMSCV), slope, bias and the ratio of standard deviation of the nutrients measured to the RMSECV (SD/RMSECV) for the validation dataset. Accurate prediction is shown by high R^2, low RMSECV, slope close to one, bias close to zero, and high SD/RMSECV.

6.2.6. Martens' Uncertainty Test

To assess which wavelengths were significantly important for the pasture nutrient predictions models a Martens" Uncertainty Test, available in The Unscrambler® software, was applied. Under cross-validation, a number of sub-models are created which are based on all the samples that were not kept out in the cross-validation segment. For every sub-model, a set of model parameters (including B-coefficients) is calculated. Variations over these sub-models will be estimated so as to assess the stability of the results. For each variable the difference between the B-coefficient B_i in a sub-model and the B_{tot} for the total model is calculated. The Unscrambler® takes the sum of the squares of the differences in all sub-models to get an expression of the variance of the B_i estimate for a variable. With a *t*-test the significance of the estimate of B_i is calculated. Thus, the resulting regression coefficients can be presented with uncertainty limits that correspond to 2 standard deviations under ideal conditions. Variables with uncertainty limits that do not cross the zero line are significant variables. Variables that are non-significant in the Martens' Uncertainty Test display non-structured variation (i.e. noise), and they can be considered useless or unreliable variables (Martens and Martens 2000, CAMO 2007).

6.3. Results and discussion

6.3.1. Pasture chemical analysis

A wide range of concentrations were obtained for the three nutrients analysed (Table 6.2). The coefficient of variation (CV), calculated by dividing the standard deviation (SD) by the mean, was the same for P and K (0.28) and lower for N (0.21).

Table 6.2. Summary of statistics for pasture N, P and K concentration for entire dataset and for data divided into calibration and validation sets.

Nutrient (%)	Min.	Max.	Range	Mean	SD	CV
N (n = 357)	1.16	4.99	3.83	3.53	0.73	0.21
calibration (n = 179)	1.16	4.99	3.83	3.52	0.74	0.21
validation (n = 178)	1.42	4.93	3.51	3.53	0.73	0.21
P (n = 473)	0.15	0.79	0.64	0.40	0.11	0.28
calibration (n = 237)	0.15	0.79	0.64	0.40	0.11	0.28
validation (n = 236)	0.17	0.67	0.50	0.39	0.11	0.28
K (n = 376)	0.90	4.20	3.30	2.55	0.71	0.28
calibration (n = 188)	0.90	4.15	3.25	2.55	0.71	0.28
validation (n = 188)	0.90	4.20	3.30	2.56	0.71	0.28

Pasture samples analysed for N ranged in value from 1.16% to 4.99%, with an averaged value of 3.53%. According to McNaught (1970) critical N values for optimum clover and ryegrass growth are from 4.50-5.50% and 4.00-4.50%, respectively. Cornforth and Sinclair (1984) defined the optimum N concentration for white clover of 4.80-5.50% and 4.5-5.0% for ryegrass. In this study only 24% of the samples had N values over 4.50%, with the majority of these samples from two dairy pastures sites (Ruakura and Colyton), which received fertiliser N and P inputs. The lowest N values were observed for pasture samples from hill country (Alfredton and Ballantrae), sites of lower fertility.

McNaught (1970) defines critical P levels of 0.30-0.40% for optimum clover growth and 0.28-0.36% for optimum ryegrass growth, while Cornforth and Sinclair (1984) define optimum P level of 0.35% for white clover and 0.40% for ryegrass. Within the 473 pasture samples P concentration varied from 0.15% to 0.79%, with 0.40% as the mean value. Lowest P values came from samples collected at one of the hill country sites (Alfredton) and the highest values from dairy pastures receiving large amounts of P fertilizer (Colyton).

Williams (1988) established 2.0-2.5% K as being adequate for plant growth, with levels >2.5% K being luxury uptake. McNaught (1970) defined the critical K concentration for optimum ryegrass and white clover growth of 2.2% and 2.0%, respectively. Smith and Middleton (1978) in a survey of New Zealand pastures found K concentrations ranging from 0.98 to 5.21%, with 75% of the sites sampled having concentrations >2.5%. In this study, K concentrations ranged from 0.90% to 4.20%, with an average value of 2.55%. 50% of samples had K levels >2.5%, coming primarily from Tokoroa, Colyton and Ballantrae. Under 25% of samples had K values under the critical level, coming mostly from Ruakura, Atiamuri and Alfredton.

6.3.2. Prediction of pasture N, P and K concentration using different mathematically transformed spectral data

The accuracy of PLSR calibration models (calibration datasets; N: 179 samples; P: 237 samples; K: 188 samples) in predicting N, P and K concentrations of pasture from different mathematically transformed spectral data was tested using separate validation datasets (N: 178 samples; P: 236 samples; K: 188 samples) (Table 6.3).

Table 6.3. PLSR results between pasture nutrient concentration and reflectance factor (R), first derivative reflectance (FDR), absorbance (Log(1/R), first derivative of absorbance (FDLog(1/R)), continuum removed reflectance (CR), continuum removed derivative reflectance (CRDR), band depth ratio (BDR) and normalized band depth index (NBDI) for the validation datasets using regression models developed from the calibration datasets.

	Mathematical transformations	Latent Variables	R^2	RMSEP	Slope	Bias	SD/RMSEP
Nitrogen %	R	6	0.49	0.52	0.52	-0.0117	1.4
	FDR	5	0.45	0.54	0.54	-0.0229	1.4
	Log(1/R)	5	0.37	0.58	0.38	0.0121	1.3
	FDLog(1/R)	3	0.34	0.59	0.33	0.0453	1.2
	CR	8	0.40	0.56	0.46	0.0202	1.3
	CRDR	2	0.28	0.62	0.28	0.0182	1.2
	BDR	1	0.24	0.63	0.21	-0.0012	1.2
	NBDI	9	0.41	0.56	0.47	0.0269	1.3
Phosphorus %	R	11	0.53	0.07	0.59	-0.0001	1.5
	FDR	8	0.53	0.07	0.61	-0.0004	1.5
	Log(1/R)	10	0.55	0.07	0.59	-0.0008	1.5
	FDLog(1/R)	8	0.53	0.07	0.61	-0.0013	1.5
	CR	12	0.38	0.09	0.47	-0.0003	1.3
	CRDR	6	0.35	0.09	0.50	-0.0010	1.3
	BDR	2	0.20	0.10	0.26	-0.0042	1.1
	NBDI	2	0.23	0.10	0.28	-0.0011	1.2
Potassium %	R	13	0.52	0.49	0.61	-0.0773	1.5
	FDR	9	0.51	0.50	0.63	-0.0129	1.4
	Log(1/R)	9	0.44	0.53	0.48	-0.0179	1.3
	FDLog(1/R)	5	0.45	0.52	0.52	0.0220	1.4
	CR	7	0.38	0.56	0.45	-0.0315	1.3
	CRDR	5	0.38	0.56	0.46	-0.0232	1.3
	BDR	1	0.15	0.65	0.17	0.0268	1.1
	NBDI	9	0.36	0.57	0.45	-0.0797	1.3

The PLSR prediction models developed from the all-seasons dataset collected for all three nutrients were inaccurate at predicting N, P and K concentrations. Slightly better prediction results were obtained using the R, FDR, Log(1/R) and FDLog (1/R) datasets. Although previous research had reported that continuum removal analysis would be useful in predicting pasture parameters *in situ* (Mutanga *et al.* 2004, Kawamura *et al.* 2009a), the PLSR results for N, P, K predictions in this study, were not improved by the continuum removed spectra of the seven absorption features selected. This was irrespective of the method of data transformation (CR, CRDR, BDR and NBDI, Table 6.3). Results might have been improved if the continuum was calculated using different wavelength ranges (Kawamura *et al.* 2009a), but it was decided not to explore the continuum removal process further in this research. Hence for the pasture nutrient predictions in different seasons of the year analyses were restricted to the use of R, FDR, Log(1/R) and FDLog(1/R) data.

There is very little published research that has explored the prediction of pasture nutrient content by testing different mathematically transformed spectral data, and of that published, the results diverge greatly. For example Mutanga *et al.* (2004) used 96 samples collected in the Kruger National Park, South Africa, during the dry season of 2002, to predict *in situ* nutrient concentration of a pasture composed of mixed species. The data were split into training (calibration dataset, n = 72) and testing sets (validation dataset, n = 24), and using stepwise multiple linear regression (SMLR) and a bootstrapping procedure, predictions for N, P and K yielded mean RMSEP of 0.08, 0.02 and 0.05, respectively, and mean R^2 of 0.60, 0.70 and 0.53, respectively. These results were achieved by analysing CRDR data, but the authors also ran regressions for band depth (BD) (BD = 1-CR), BDR and NBDI transformations, and those resultant R^2 values were similar to our results (Table 6.3), but in their case the CRDR yielded better results. The RMSEP values for our predictions are much higher than the values presented by Mutanga *et al.* (2004), especially for N and K. Compared with the Mutanga *et al.* (2004) study, where the N concentration ranged from 0.38 to 2.00% (mean = 0.78%), P ranged between 0.04-0.48% (mean = 0.18%), and K from 0.21 to 2.71% (mean = 0.96); in this study nutrient concentrations were much higher (Table 6.2).

Bogrekci and Lee (2005) using absorbance data (between 225 and 2525 nm) and P concentration of 150 fresh Bahia grass samples collected in three sites in the Lake Okeechobee drainage basin in Florida, obtained the best result (R^2 = 0.43 and RMSEP = 0.07%) using PLSR compared to SMLR. The values of RMSEP are similar to those for P% in this study, but the R^2 was higher. Again compared with the present study, the P% in the Bahia grass samples were much lower (0.12-0.50%).

Under controlled conditions, and in one season, Mutanga *et al.* (2005) used 96 *Cenchrus ciliaris* grass plots from a greenhouse experiment to predict pasture quality using continuum removal analysis in the visible domain (550-750 nm). SMLR and bootstrapping procedures were applied to transformed spectral data (CRDR, BD, BDR and NBDI) and pasture nutrient concentration. Using R^2 to test the model‟s accuracy for predicting pasture N and K concentrations, the highest R^2 (0.73 and 0.33, respectively) were obtained with

NBDI data, while for P concentrations the best result (R^2 = 0.33) was achieved with BDR data. Apart from the high accuracy of the N calibration model, these findings are similar to the findings of the present study from sampling contrasting field sites *in situ* in different seasons.

Thulin *et al.* (2006) predicted *in situ* pasture crude protein (CP) of 75 samples collected on December 2000, January and October 2002, from two sites in the south-eastern temperate zone of Victoria, Australia. Using PLSR full cross-validation, the best results were obtained using derivative of absorbance data (FDLog(1/R)) and yielded a R^2 = 0.61 and a RMSECV of 2.97% CP (equivalent to 0.48% N, since CP is 6.25 times the nitrogen content of forages). The data collected in 2000 (n = 30) and in 2002 (n = 45) were captured using different methods, with the former having an internal average of 10 captured directly above a pasture canopy. The latter had an internal averaging of 50 or 200 and consisted of multiple spectra averaged over an area. Limiting analysis to samples collected in January and October 2002 improved the relationship (R^2 = 0.79 and RMSECV = 2.39% CP (0.38% N)).

Kawamura *et al.* (2009a) used 96 samples (48 samples for calibration and validation) to estimate New Zealand hill pasture nutrient content. Reflectance, derivatives and continuum-removed reflectance data were regressed against pasture nutrient content (in kg ha^{-1}) using PLSR. Best predictions for N (R^2 = 0.89) and P (R^2 = 0.94) content were obtained when analysing CRDR data, and best K content prediction (R^2 = 0.81) when analysing FDR. The analyses of Kawamura *et al.* (2009a) were limited to data collected from a single farm on a single date (during spring-summer time transition), which may account for the improved accuracy of their calibration models.

6.3.3. Predictions of pasture nutrient concentration by season

The ability to predict pasture nutrient concentration year round from one calibration offers the most flexibility, but restricting the calibration and validation of the PLSR model to one season might improve the accuracy of the model. It may be that improved accuracy at critical times of the year has greater utility as reported by Mutanga *et al.* (2004) who focused their pasture quality prediction study at the beginning of the dry season, because nutrient quality became more critical at this time of the year.

6.3.3.1. Nitrogen

On average the winter pasture samples had the highest N concentration and spring samples the lowest with the greatest range of N concentrations measured in summer (from severely N deficient values of 1.16% N to an adequate N concentration of 4.99%, Table 6.4).

Table 6.4. Summary of statistics for pasture N concentration (%) per season.

		Number			Nitrogen			
Season	Site	of samples	Min	Max	Range	Mean	SD	CV
Spring	Alf., Bal., Col., Man., Ati.	162	1.69	4.93	3.24	3.34	0.64	0.19
Summer	Alf., Bal., Rua.	82	1.16	4.99	3.83	3.73	0.88	0.24
Autumn	Alf., Col., Ati.	61	1.42	4.42	3.00	3.38	0.72	0.21
Winter	Alf., Col.	52	2.75	4.85	2.10	3.94	0.49	0.13

Alf. – Alfredton, Bal. – Ballantrae, Col. – Colyton, Man. – Manawahe, Rua. – Ruakura, Ati. –Atiamuri.

The accuracy of the PLSR calibration models differed little with the four types of transformed spectral data (Table 6.5, Figure 6.2). The models derived for the spring data presented the lowest R^2s (average R^2 = 0.43) in comparison with the other three seasons (average R^2 = 0.76). The coefficient of variation (CV) can impact on the predictive capacity of the data regressions, but interestingly even with a low CV (0.13) the winter samples reached the highest R^2 observed (average R^2 = 0.78). Conversely, the regressions for spring yielded very low values of R^2, but that can not be explained by the low CV (0.19).

Table 6.5. Seasonal summary of prediction statistics (PLSR full cross-validation) for pasture N% using R, FDR, and Log(1/R) data between 420-2400 nm.

	Latent variables	R^2	RMSECV	Slope	Bias	SD/RMSECV
Spring						
R	8	0.43	0.49	0.46	0.0063	1.3
FDR	8	0.43	0.48	0.53	0.0034	1.3
Log(1/R)	9	0.49	0.46	0.55	0.0028	1.4
FDLog(1/R)	4	0.37	0.51	0.43	0.0028	1.3
Summer						
R	7	0.72	0.47	0.76	-0.0015	1.9
FDR	8	0.78	0.41	0.83	-0.0030	2.1
Log(1/R)	8	0.74	0.45	0.77	0.0089	2.0
FDLog(1/R)	8	0.76	0.43	0.77	0.0089	2.0
Autumn						
R	3	0.77	0.35	0.79	-0.0011	2.0
FDR	3	0.75	0.36	0.77	-0.0012	2.0
Log(1/R)	4	0.75	0.36	0.78	0.0018	2.0
FDLog(1/R)	3	0.77	0.35	0.80	-0.0011	2.1
Winter						
R	5	0.75	0.25	0.80	0.0028	2.0
FDR	5	0.79	0.23	0.86	-0.0031	2.2
Log(1/R)	5	0.74	0.25	0.81	-0.0093	2.0
FDLog(1/R)	4	0.83	0.21	0.84	0.0100	2.4

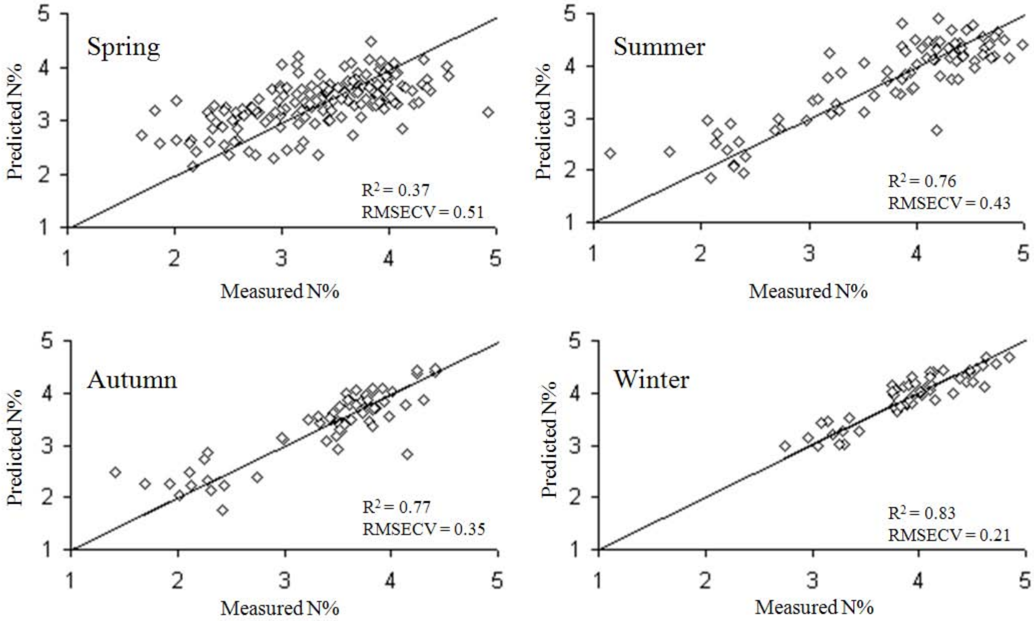

Figure 6.2. Relationships between measured pasture nitrogen concentrations and those predicted from the FDLog(1/R) dataset for the four seasons of the year.

Considering all the statistics presented for the PLSR models (Table 6.5) the most accurate predictions were obtained for the winter samples and the least accurate predictions for spring samples. There are major pasture-related differences between these two seasons including photosynthetic activity and rate of growth and nutrient concentration (Table 6.4).

Based on an interpretation of SD/RMSECV values (Table 6.5), calibration models for pasture N% prediction *in situ* for summer (exception for R data), autumn and winter were reasonably accurate, but inaccurate for spring. Of the data collected in spring, the smallest average difference between predicted N values (by PLSR model) and measured N values was observed for samples collected from Colyton in September 2007 (average difference between measured and predicted N values significantly smaller (*t*-test, $p < 0.05$) than the average difference of all other sites). This dataset, Colyton September 2007, was collected during early spring, while all other datasets examined were collected during mid or late spring. This difference in time of sampling may have been important because, in early spring, grasses have vigorous leaf production, whereas in mid and late spring, as the temperatures rise, the proportion of stem and inflorescence increases (Chaves 2003).

In comparison to the Colyton September 2007 (early spring) dataset, the dataset collected at Colyton in October 2006 (mid spring) presented one of the highest average differences between measured and predicted N values, 0.20 and 0.48 (*t*-test $p < 0.05$) respectively. Although the reproductive components of the herbage samples collected were not quantified at this site, pictures taken of the Colyton plots in October 2006 indicate a similar proportion of stems and inflorescences to that seen at the same site in September 2007. Standing biomass was on average higher (20%) in October 2006 (range = 1985-3360 kg DM/ha, average = 2642 kg DM/ha) than September 2007 (range = 1559-2807 kg DM/ha, average = 2123 kg DM/ha). While there was no significant correlation (*t*-test, $p < 0.05$) between standing biomass and the differences between predicted and measured N values for the pasture samples collected during September 2007, a negative correlation of 0.70 (*t*-test, $p < 0.05$, data not shown) was observed for October 2006 samples. In general, two distinct trends were observed in the October 2006 dataset, overall the samples with biomass up to approximately 2500 kg DM/ha had the N% overestimated by the PLSR models, while samples with biomass greater than 2500 kg DM/ha had the N% underestimated by the models.

While the Colyton pasture plots were cut and not grazed by animals, at Manawahe (range = 927-2096 kg DM/ha, average = 1421 kg DM/ha) and Alfredton (range = 1197-2376 kg DM/ha, average = 1759 kg DM/ha) pastures were under dairy and sheep grazing, respectively. For Manawahe a low correlation ($r = -0.39$, $P < 0.05$) was observed between the biomass and the differences between predicted and measured N values, while at Alfredton no statistically significant correlation ($P < 0.05$) was observed.

The N predictions accuracy for summer, autumn and winter samples (Table 6.5) were improved compared to the regressions based on all-seasons data (Table 6.3). If the spring data are excluded from the all-seasons dataset and a new regression is done (PLSR using cross-validation and R data) ($n = 195$) the result (LV = 15, $R^2 = 0.78$, slope = 0.85, bias = 0.0006, RMSECV = 0.37, and SD/RMSCV = 2.1) is improved (c.f. all seasons data in Table 6.3) and is comparable with the three individual seasons analysed separately (Table 6.5). Regressions using absorbance and derivatives data against summer, autumn and winter samples combined had inferior results to the one obtained using the reflectance factor (data not shown).

In New Zealand pastoral systems, N fertiliser is still used predominantly to lift pasture production in periods of low growth, which are commonly caused by seasonally low soil temperatures. N fertiliser is not commonly used to sustain all year round base pasture production. N supply for base productions is achieved through the inclusion of N fixing legumes in the pasture mix. New Zealand pastures, however, are responsive to applied N fertiliser in all months of the year (Lynch 1982), although the greatest use would be the autumn and winter and early spring months. Lynch (1982) suggests that the ability to predict the likelihood of a pasture growth response to N applied in autumn is poor. Compared with the spring, pasture growth in the autumn and winter can be limited by a number of factors (e.g. temperature, moisture and mineralizable N levels), all of which will affect the response to added N. Improving the ability to predict likelihood and size of the pasture response to added N in the autumn and winter by sensing the N concentration in the mixed pasture would have immediate economic benefits by avoiding some unnecessary fertiliser application. In turn this would reduce pasture N concentrations leading to less urine N and lower gaseous and leaching losses of N to the environment. The results presented in this Chapter show that the N concentration (degree of N deficiency) in New

Zealand pastures could be predicted with reasonable accuracy using the spectral reflectance of pasture samples taken in summer, autumn and winter seasons. The ability to accurately sense pasture for N concentration immediately before applying N using hand held sensors could result in significant saving in N fertiliser use and improvements to the environment.

6.3.3.2. Phosphorus

The highest herbage P concentrations occurred in spring samples; followed by summer, winter and autumn samples (Table 6.6). The greatest range in P concentrations also occurred in spring (from severely P deficient values of 0.19% P to an excessive P concentration of 0.79%). Summer and autumn samples had similar average P concentrations (0.34), likewise did winter and spring samples (0.43). The CV was similar for spring, summer and autumn; and lowest in winter (0.12).

Table 6.6. Summary of statistics for pasture P concentration (%) per season.

Season	Site	Number of samples	Min	Max	P Range	Mean	SD	CV
Spring	Alf., Bal., Col., Man., Tok., Ati.	210	0.19	0.79	0.60	0.43	0.12	0.28
Summer	Alf., Bal., Rua.	105	0.20	0.62	0.42	0.34	0.09	0.27
Autumn	Alf., Col., Ati.	84	0.15	0.52	0.37	0.34	0.10	0.29
Winter	Alf., Col.	74	0.33	0.56	0.23	0.43	0.05	0.12

Alf. – Alfredton, Bal. – Ballantrae, Col. – Colyton, Man. – Manawahe, Rua. – Ruakura, Tok. – Tokoroa, Ati. –Atiamuri.

The PLSR calibration models predicted pasture P concentration to a similar level of accuracy irrespective of the method of data transformation used (Table 6.7, Figure 6.3). All PLSR models using observations taken in winter were inaccurate (very low R^2) at predicting unknown herbage P concentrations. The inaccuracy is attributed to the very low range of P concentrations observed in winter samples (0.23) and low CV (0.12). PLSR calibration models based on spring observations were moderately accurate (R^2s 0.53 - 0.60) for all sites, although spring data had the highest range and second highest CV. The most accurate PLSR calibration models were produced from autumn observations (highest R^2 and highest SD/RMSECV).

Table 6.7. Seasonal summary of prediction statistics (PLSR full cross-validation) for pasture P% using R, FDR, and Log(1/R) data between 420-2400 nm.

	Latent variables	R^2	RMSECV	Slope	Bias	SD/RMSECV
Spring						
R	7	0.53	0.08	0.56	0.0005	1.5
FDR	7	0.59	0.08	0.63	0.0002	1.6
Log(1/R)	10	0.60	0.08	0.64	0.0000	1.6
FDLog(1/R)	5	0.60	0.08	0.64	0.0008	1.6
Summer						
R	7	0.65	0.05	0.68	-0.0007	1.7
FDR	7	0.74	0.05	0.77	-0.0004	1.9
Log(1/R)	5	0.65	0.05	0.68	0.0002	1.7
FDLog(1/R)	5	0.70	0.05	0.73	-0.0002	1.8
Autumn						
R	3	0.76	0.05	0.76	-0.0001	2.0
FDR	2	0.77	0.05	0.77	-0.0001	2.1
Log(1/R)	2	0.75	0.05	0.75	-0.0002	2.0
FDLog(1/R)	1	0.76	0.05	0.76	-0.0001	2.1
Winter						
R	3	0.17	0.05	0.18	-0.0001	1.1
FDR	3	0.14	0.05	0.02	-0.0001	1.1
Log(1/R)	2	0.12	0.05	0.14	-0.0002	1.1
FDLog(1/R)	2	0.16	0.05	0.21	-0.0003	1.1

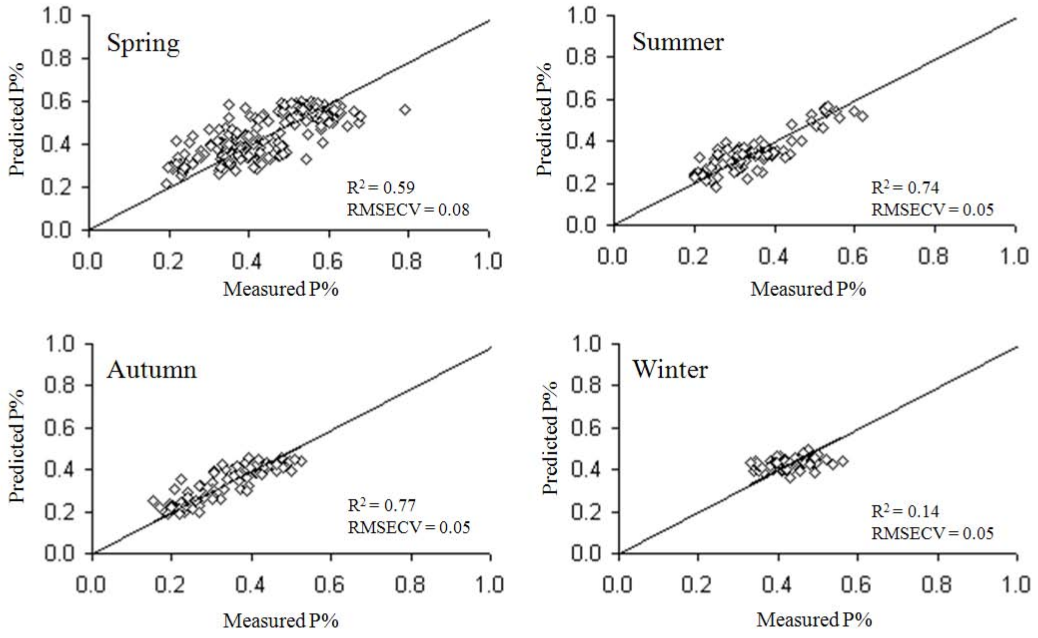

Figure 6.3. Relationships between measured pasture phosphorus concentrations and those predicted from the FDR dataset for the four seasons of the year.

PLSR calibration models produced using summer, autumn and winter observations had the same error of prediction (RMSECV = 0.05) but because the SD of winter samples (0.05) is about half of the SD of summer (0.09) and autumn (0.10) samples, the comparative level of accuracy of prediction (SD/RMSECV ratio) for winter samples is half that of the summer and autumn values. This indicates that less accurate predictions result from samples sets with small ranges rather than the inability of the methodology (field hyperspectral data) to predict the nutrient content.

PLSR models based on observations taken in autumn samples followed by summer produced the most accurate predictions (Table 6.7). When the summer and autumn samples were pooled, a PLSR calibration produced using FDR data had slightly lower R^2 and SD/RMSECV values (LV = 8, $R^2 = 0.69$, slope = 0.79, bias = -0.0001, RMSECV = 0.05, and SD/RMSECV = 1.8) compared to the regressions for summer and autumn data separately (Table 6.7); but greater accuracy compared to the regression for all seasons combined (Table 6.3).

The PLSR calibration models presented above showed that reasonably accurate pasture P concentration predictions could be achieved in autumn. In New Zealand, P fertilisers are applied annually to pastures, to maintain or increase pasture production by encouraging legume growth (and therefore N fixation) (Roberts and Morton 1999). Most annual P fertiliser applications are made in autumn when dry soil conditions allow the operation of heavy spreading machinery. Soil P testing has traditionally been used for monitoring the P status of the pasture, although pasture P concentrations are also used to monitor the P status. Monitoring by either soil testing or collecting pasture samples for testing in the laboratory is generally limited to a few fixed sites on a pastoral farm. These fixed sites are then used to provide an indication of the likely P levels over much wider areas. The findings of the present study show that hyperspectral remote sensing can predict pasture P in autumn with reasonable accuracy, offering an approach that would allow the P concentration in pasture to be measured beyond fixed transects, providing an indication of the changes in P status for example in and around water ways and within and across paddocks, opening the opportunity for increasing the efficiency of use of applied P and reducing P losses to the environment.

6.3.3.3. Potassium

The greatest range of K concentrations occurred during summer, followed by spring, autumn and winter (Table 6.8). The K concentrations in New Zealand pastures have been previously reported to have a seasonal variation, with the concentration lower in the drier months of the year and higher in the wetter months (Williams 1988; Thomas and Hipp 1968). Our findings agree with this; with the highest average K concentrations occurring in spring and winter (wetter months), than in summer and autumn samples (drier months).

Table 6.8. Summary of statistics for pasture K concentration (%) per season.

Season	Site	Number of samples	Min	Max	K Range	Mean	SD	CV
Spring	Alf., Bal., Col., Man., Tok., Ati.	158	1.50	4.20	2.70	3.02	0.55	0.18
Summer	Alf., Bal., Rua.	93	1.00	4.15	3.15	2.17	0.67	0.31
Autumn	Alf., Col.	63	0.90	2.76	1.86	1.87	0.46	0.25
Winter	Alf., Col.	62	1.73	3.40	1.67	2.62	0.36	0.14

Alf. – Alfredton, Bal. – Ballantrae, Col. – Colyton, Man. – Manawahe, Rua. – Ruakura, Tok. – Tokoroa, Ati. –Atiamuri.

The PLSR calibration models built using the FDR data from the summer and autumn samplings were slightly more accurate than the PLSR models using the other spectral data transformations (Table 6.9, Figure 6.4). The only accurate models were produced using summer observations (R^2 values ≥ 0.7, RMSECV ≤ 0.37, SD/RMSECV ≥ 1.8). PLSR models using spring, autumn and winter observations were less accurate (R^2 ≤ 0.40; RMSECV ≥ 0.36, SD/RMSECV ≤ 1.3). PLSR models based on winter samples had extremely low R^2s, which can be explained by a low sample values range and the lowest CV among all samples.

Table 6.9. Seasonal summary of prediction statistics (PLSR full cross-validation) for pasture K% using R, FDR, and Log(1/R) data between 420-2400 nm.

	Latent variables	R^2	RMSECV	Slope	Bias	SD/RMSECV
Spring						
R	8	0.37	0.44	0.44	0.0009	1.3
FDR	4	0.33	0.45	0.36	0.0018	1.2
Log(1/R)	13	0.36	0.44	0.51	0.0026	1.2
FDLog(1/R)	5	0.32	0.46	0.46	0.0087	1.2
Summer						
R	7	0.70	0.37	0.74	-0.0045	1.8
FDR	7	0.76	0.33	0.81	-0.0033	2.0
Log(1/R)	9	0.72	0.36	0.78	-0.0006	1.9
FDLog(1/R)	5	0.73	0.35	0.75	0.0015	1.9
Autumn						
R	3	0.34	0.38	0.37	0.0024	1.2
FDR	4	0.40	0.36	0.44	0.0006	1.3
Log(1/R)	2	0.30	0.39	0.32	0.0028	1.2
FDLog(1/R)	2	0.34	0.38	0.38	0.0008	1.2
Winter						
R	1	0.01	0.36	0.01	0.0006	1.0
FDR	1	0.01	0.36	0.01	-0.0004	1.0
Log(1/R)	1	0.06	0.37	-0.02	0.0011	1.0
FDLog(1/R)	1	0.01	0.36	0.01	0.0005	1.0

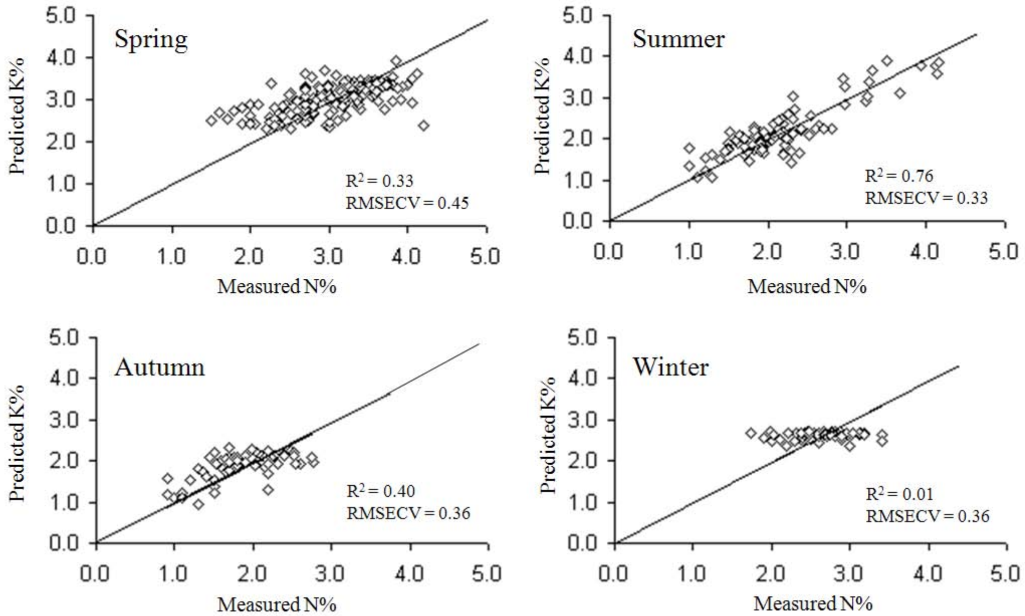

Figure 6.4. Relationships between measured pasture potassium concentrations and those predicted from the FDR dataset for the four seasons of the year.

This study shows that pasture K prediction could be achieved with reasonable accuracy for samples collected during the summer months only. In New Zealand this is one of the seasons with the lowest concentrations of K in pasture. With fertiliser K applied predominantly in late winter and early spring on NZ dairy systems to limit the loss of K by leaching and also to limit the impact of added K on pasture Ca and Mg concentrations and cow metabolism, a model calibrated from summer data would only be useful if low K values in summer coincided with low pasture K concentrations in winter and spring. Good correlations exist between summer and spring pasture K concentrations (0.64 for Alfredton site, 0.88 for Ballantrae, and 0.77 considering Alfredton and Ballantrae), which indicate that the assessment of K status could be conducted during summer with the application of K fertiliser done later in the following early spring based on the summer assessment. No correlation was observed between summer and winter samples collected at Alfredton, the only site in this study which had samples from those two seasons.

6.3.3.4. Most important wavelengths for the pasture nutrient predictions

In Figures 6.5, 6.6 and 6.7, regression coefficient plots for PLSR calibration models are shown for the prediction of N% (using the FDLog(1/R) data), and for P% and K% (using FDR datasets). Regression coefficients of the PLSR calibration models indicate the importance of a particular waveband in explaining the variance in the measured pasture N, P and K concentrations (Figures 6.5, 6.6 and 6.7).

The wavelengths detected as significant by the Martens" Uncertainty Test are highlighted in the regression coefficient plots (Figure 6.5, 6.6 and 6.7). Some wavelengths which presented high values of regression coefficients were detected as non-significant (useless or unreliable) in the Martens" Uncertainty Test; these wavelengths were mainly localized in the extremes parts of the spectrum and have higher noise (low signal-to-noise ratio).

For the prediction of pasture N, wavebands in the visible (Vis) part of the electromagnetic spectrum was the most important for the models, independent of the season analysed (Figure 6.5). Chlorophyll absorption bands are found at 430 nm, 460 nm, 640 nm and 660 nm; and since N is a key component of chlorophyll, different levels of plant N are expected to be seen as a different concentration of plant chlorophyll (Donahue *et al*. 1983).

The red edge (680-750 nm) has been reported to have a good correlation with N content and other biochemical compounds (Lichtenthaler *et al.* 1996, Lamb *et al.* 2002, Mutanga *et al.* 2005). In the present study better predictions of herbage N% were achieved for the seasons where red edge wavelengths had greatest influence on the prediction model. However, N absorption features for dried and ground vegetation samples are found not only at the previously mentioned chlorophyll absorption bands but also at 910 nm, 1020 nm, 1510 nm, 1690 nm, 1940 nm, 1980 nm, 2060 nm, 2130 nm, 2180 nm, 2240 nm, 2300 nm, 2350 nm (Curran 1989). These are protein absorption features which are of course closely related to N% content. It is well known that one of the challenges when working with spectra of fresh leaves is that water in the leaves masks absorption features (Elvidge 1990), especially between 1300-2500 nm where water strongly absorbs energy (Carter 1991). The fact that wavelengths in the Vis are less influenced by water than in infrared (IR) might explain why the most important wavelengths for N prediction of fresh pasture canopies in this study were found in the Vis region.

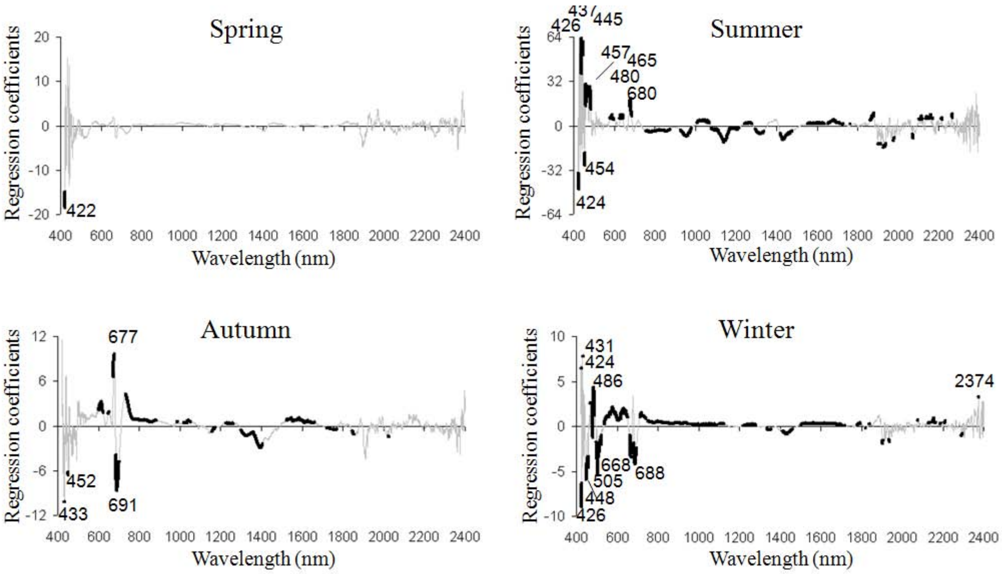

Figure 6.5. Regression coefficients of PLSR models for pasture N% prediction using the FDLog(1/R) dataset by season. Plotted in black are the significant wavelengths for the regression according to the Martens‟ Uncertainty Test. Values plotted in the graphs represent the significant wavelengths with highest regression coefficient values.

When using the FDLog(1/R) data to calibrate the PLSR model, the most accurate results for predicting N% were achieved with winter observations, followed by autumn and summer, while spring observations yielded inaccurate results (Table 6.5). The Uncertainty test revealed that for the spring dataset, only two wavelengths (421 nm and 422 nm) out of the 1981 wavelength bands analysed were considered significant in the PLSR model. For winter, summer and autumn datasets 963, 822 and 595 wavelength bands spread all over the spectrum range analysed were significant, respectively.

The most influential wavelengths for the PLSR models used to predict P% (Figure 6.6), were spread all over the Vis and IR wavelength regions. The number of wavelength bands considered significantly important by the Uncertainty Test was 1691, 753, 454 and 383 for autumn, spring, winter and summer, accordingly. Five distinct wavebands, localized at around 732-735 nm, 948-949 nm, 1134-1141 nm, 1403-1408 nm and 1884-1886 nm, were the most important wavelengths for prediction of P% during autumn (using FDR data, Table 6.7). The PLSR model built using winter data had only 3 highly important wavelength ranges, 709-710 nm, 1390-1392 nm and 1872-1873 nm. For summer, the wavelength at 420 nm had the highest absolute regression coefficient value with much smaller regression coefficients associated with other wavelengths. For spring, 5 wavelengths presented the highest values of regression coefficients with much smaller regression coefficients associated with other wavelengths.

Seasonal variations in the important wavelengths selected by the PLSR calibration models to predict pasture nutrient content were also reported by Kawamura *et al.* (2009b). In their study reflectance data of 30 pasture plots measured in two seasons were used to determine the best paired combination of bands (as input to normalized difference spectral indices) for predicting pasture P. The frequency distribution of selected wavebands changed with season, and in spring Vis wavelengths were mostly selected while in summer wavelengths in both Vis and IR regions were important.

The most accurate predictions of pasture P content in this study were achieved for the samples collected during the drier months (autumn and summer) than for wet months. One explanation for this could be the pasture samples collected during wet months have higher moisture contents with more pronounced spectral absorption bands at 1200 nm, 1450 nm and 1940 nm, which are central wavelengths of water absorption bands (Curran

1989), which are close to bands associated with prediction of P concentration in this study (e.g. near water absorption band 1450 nm, the wavelengths between 1403-1408 nm had significant influence on the autumn PLSR model, and for winter, the range was between 1390-1392 nm).

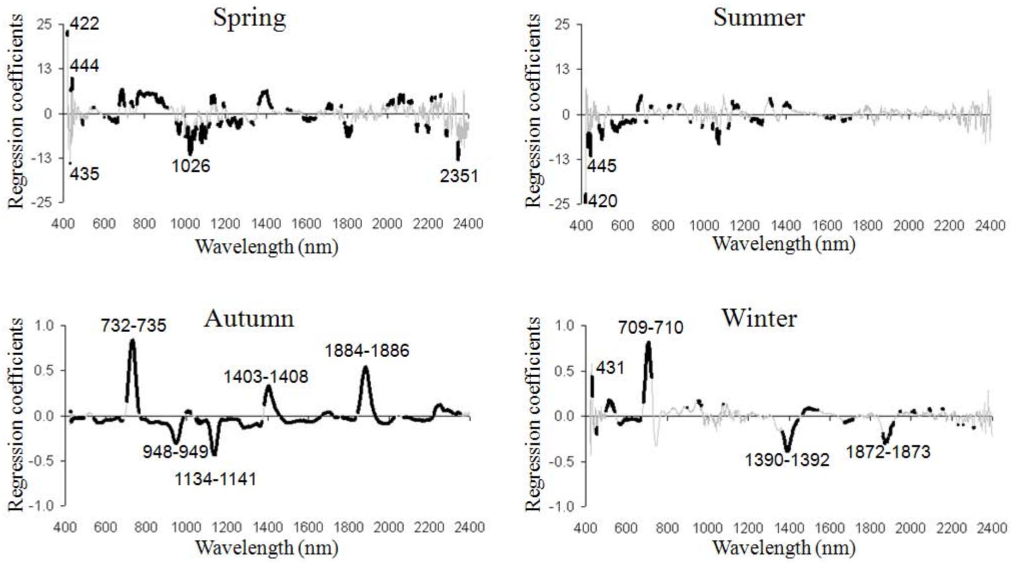

Figure 6.6. Regression coefficients of PLSR models for pasture P% prediction using the FDR dataset per season. Plotted in black are the significant wavelengths for the regression according to the Martens‟ Uncertainty Test. Values plotted in the graphs represent the significant wavelengths with highest regression coefficient values.

The regression coefficient plots for K prediction varied significantly among seasons. According to the Uncertainty Test, spring, summer, autumn and winter PLSR calibration models (using FDR data) utilised 814, 484, 127 and 100 significantly important wavelength bands, respectively (Figure 6.7). The most accurate predictions of K concentration were produced using the summer observations (Table 6.9), and the most influential wavelength for this regression model was observed at 420 nm, followed by other visible wavelengths. Kawamura *et al.* (2009b), analysing the best paired combination of bands as input to normalized difference spectral indices, also found that visible wavelengths were mostly important for predicting pasture K concentrations in spring. For the summer datasets wavelengths from both visible and IR regions were important.

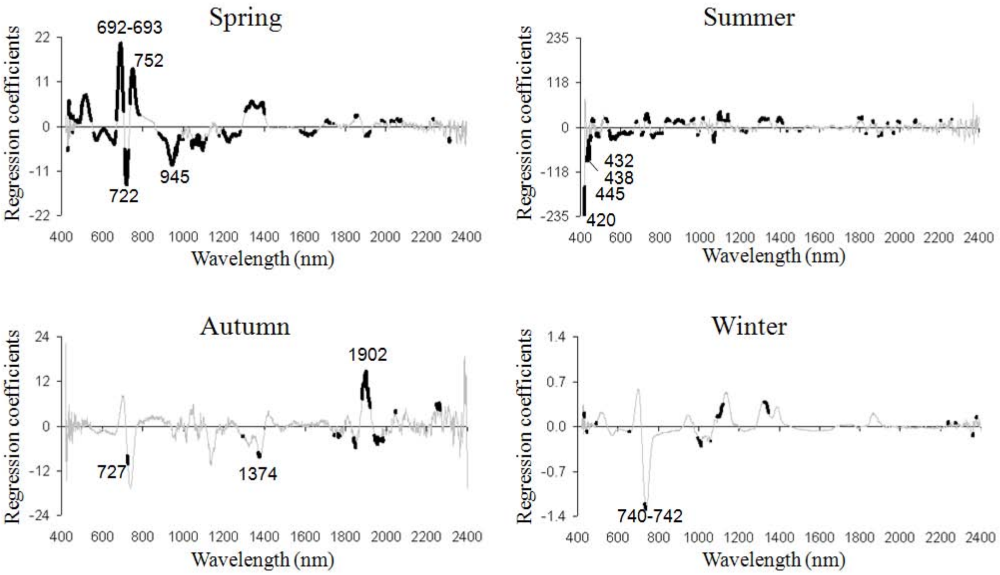

Figure 6.7. Regression coefficients of PLSR models for pasture K% prediction using the FDR dataset per season. Plotted in black are the significant wavelengths for the regression according to the Martens‟ Uncertainty Test. Values plotted in the graphs represent the significant wavelengths with highest regression coefficient values.

Overall, the best prediction accuracy for pasture P and K concentration occurred during the drier seasons (autumn and summer). For predicting N concentration season was less important, although in spring months when pastures were growing the most actively the prediction was inaccurate. The wavelengths with higher values of regression coefficients for N prediction were localized mainly in the visible region of the spectrum, where water has less influence. For P and K predictions wavelengths of great significance were found in both visible and IR regions. There were indications that the water content of the pasture samples may influence the accurate prediction of pasture P and K concentrations more than pasture N concentrations.

6.4. Conclusions

The present study has shown that there is potential to predict pasture macro-nutrient concentrations using proximally sensed hyperspectral reflectance from the pasture. Clustering the data according to the season of the year increased the accuracy of predicting the concentrations of the three pasture macro-nutrients. The best predictions were obtained with first derivative spectral data, for N% during winter (R^2 = 0.83, RMSECV = 0.21, slope

= 0.84, bias = 0.0100 and SD/RMSECV = 2.4), although autumn and summer datasets also produced reasonable predictions; for P% during autumn (R^2 = 0.77, RMSECV = 0.05, slope = 0.77, bias = -0.0001 and SD/RMSECV = 2.1); and for K% during summer (R^2 = 0.76, RMSECV = 0.33, slope = 0.81, bias = -0.0033 and SD/RMSECV = 2.0). Pastures samples collected during winter presented the lowest range of nutrient concentrations, which might explain the low accuracy of predicting P% and K% for this season. Calibrations models derived from spring data were in general inaccurate. The reason for that is not fully understood and further research is necessary to explain it.

Even though the prediction accuracy presented in this research might not be as high as that reported for laboratory NIR spectroscopy on dried, ground herbage samples, it has demonstrated that reasonably accurate predictions can be attained from fresh *in situ* canopy reflectance measurements.

CHAPTER 7:

Estimation of nutrient concentration and mass of pastures grown under different soil phosphorus status and varying nitrogen fertiliser regimes using field spectroscopy

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Some results of this study have been orally presented and published (full paper) in the proceedings of the 14th Australasian Remote Sensing and Photogrammetry Conference, Darwin, Australia, 29-03 October 2008.

Abstract

The potential of field spectroscopy to estimate phosphorus (P) and nitrogen (N) content of pasture swards *in situ*, for ensuring optimum use of these two nutrients in grazed pastures, was evaluated by collecting spectral data from a field study examining the influence of N fertiliser on a legume-based pasture response to added P fertiliser. Spectral data between 350-2500 nm were collected at four dates from 40 pasture plots which had received applications of P (*added P*) and P and N (*added P+N*) fertilisers. Calibration models were developed using partial least squares regression (PLSR) from first derivative reflectance (FDR) and measured pasture P and N concentration (%) and mass (kg ha^{-1}). The accuracy of the models was tested using leave-one-out cross-validation. Pasture P concentration and mass were inaccurately predicted (R^{2}s = 0.31-0.42 and SD/RMSECV = 1.2-1.3). Higher R^{2}s and SD/RMSECV resulted for N prediction when the regressions were carried out using the nutrient mass (R^{2}s = 0.61-0.78 and SD/RMSECV = 1.6-2.1) rather than nutrient concentration (R^{2}s = 0.39-0.63 and SD/RMSECV = 1.3-1.6). The prediction accuracy for N was better for the *added P* pasture compared to the *added P+N* pasture, and when separate models were used instead of a regression using all samples. This highlights the importance of knowing the sample set"s content when building, evaluating and using prediction calibrations. Estimates of R^{2}s and SD/RMSECV for pasture potassium, calcium, magnesium, sodium and sulphur are also presented in the paper. Confidence in using a single model to predict multiple nutrients, or a model developed for individual nutrients, will only come through building a good understanding of the factors likely to influence any one calibration function.

Keywords: minerals, field measurements, pasture sward, hyperspectral data, remote sensing

7.1. Introduction

Soils in New Zealand generally have pHs < 6.0 and are naturally deficient in nitrogen (N) and phosphorus (P), with sulphur (S) deficiency widespread and potassium (K) deficiency being induced under intensive livestock farming (e.g.. dairying) and, or, where silage or hay is regularly harvested. The main input of N in New Zealand pastures comes from biological N fixation by legumes, with N fertilisers used to augment N supply during periods of the year when legume growth and N fixation is low. Optimum legume growth and consequently N fixation, requires soil P and S levels be maintained in the optimum range by applying P and S fertilisers (During 1972). Although P, S, K and N are the main growth limiting nutrients considered in fertiliser application, the adequacy of other nutrients (Ca, Mg and Na) also need to be monitored, including trace elements (Co, Se, Mo and Cu) for both plant growth and the dietary requirements of ruminants (Grace 1983, Chiy and Phillips 1996).

Laboratory based wet chemical methods to measure plant nutrient concentrations have been substituted by near infrared reflectance spectroscopy (NIRS), allowing quicker and cheaper estimation of forage nutrient content (Berardo 1997 and Corson *et al.* 1999). While NIRS represents an advance on wet chemical methods, there is still the requirement to collect plant samples from the field, and to dry and grind them before analysis. Direct application of spectroscopic technology *in situ* to estimate forage nutrient contents (Mutanga *et al.* 2004, Sanches *et al.* 2006 and Kawamura *et al.* 2006) has the challenge of dealing with variation in leaf internal structure, atmospheric and background effects (Yoder and Pettigrew-Crosby 1995) but has the potential not only to eliminate laboratory handling and preparation costs but also provide producers with real time data for decision making.

Field spectroscopy adds the opportunity to extend sampling beyond fixed locations or transects to the whole paddock and even the whole farm.

The biggest single expenditure faced by most New Zealand pastoral farmers is P fertiliser. Phosphorus losses from pastoral systems are implicated increasingly in declining water quality and in eutrophication of fresh water bodies. Field spectroscopy could be used to increase the precision in calculating P requirements and targeting P applications. It may also assist in identifying critical source areas enriched in P at risk of contributing P to transmission pathways to surface water bodies.

To overcome seasonal N deficiency urea is often applied at short notice to a few paddocks to boost pasture growth rates (Gillingham *et al.* 2008). Field spectroscopy could provide an estimate of the N content of pasture immediately before N fertiliser application. This has the potential to increase efficiency of N fertiliser use and to ensure N contents are not elevated above the optimum range. In doing so this would limit the loss of N leached or emitted as nitrous oxide from urine patches, as there is a direct relationship between dietary N, urine N and N loss to the environment (Ledgard *et al.* 2008).

NIRS analysis has been used to measure several constituents and properties of forages, but unfortunately the estimation of forage minerals by NIRS can be hindered by mineral concentrations with narrow ranges unsuited for calibration against spectral properties (Roberts *et al.* 2004). For estimation of leaf biochemistry (e.g. plant pigment and protein content) using NIRS, the spectral data is normally calibrated against the concentration of the biochemical represented as percentage of dry weight. However, in a study to evaluate the potential of remote sensing for estimating the biochemistry of dry and fresh leaves, Jacquemoud *et al.* (1996) pointed out that concentration might not be the most suitable unit for this application, because fractions do not represent the amount of matter interacting with light. In their study, the authors reported an increase in biochemical variation by a factor between 1 and 10 when the concentration data were expressed per unit surface area (g cm^{-2}). Datt (1998), when using leaf pigments both expressed in mg/g fresh leaf mass and in mg/cm^2 leaf area to estimate chlorophyll and carotenoid of eucalyptus leaves by remote sensing, found that while the two units differed by as much as 2 orders of magnitude, their coefficients of variation (CV) were similar. Kawamura *et al.* (2009a) in predicting herbage biomass and nutrient (N, P, K and S) contents in hill pastures reported an increase in biochemical variation (CV) two or three times larger when the data were expressed as amount per unit area (nutrient mass) instead of percent of dry weight. Accordingly, prediction calibrations based on forage nutrients expressed as amount per unit area rather than nutrient expressed as concentration might improve the estimation of nutrients in forages.

The objective of this research was to evaluate the use of field spectroscopy to estimate pasture P and N using spectral data collected *in situ* from pasture plots in a field study examining the influence of the addition of fertiliser N on a legume based pasture response to added P. The opportunity was also taken in the study to estimate the pasture K, S, calcium (Ca), magnesium (Mg) and sodium (Na) concentration and mass from the same spectral data collected. Spectral data were calibrated against measured pasture nutrient expressed as concentration (percent of dry matter) and nutrient mass per hectare (nutrient concentration (%) multiplied by the dry matter yield (kg ha^{-1})).

7.2. Materials and methods

7.2.1. Study area

The field trial site was located on a dairy farm at Colyton (40°13'S, 175°38'E), in the North Island of New Zealand. The soil of the area is classified as a Pallic soil (Perch-gley argillic pallic) (Hewitt 1993). Based upon temperature and rainfall, this site is in the mid central zone, which is characterized by few extremes of temperature and occasional droughts in summer; with pasture mostly composed of temperate grasses (Stewart and Charlton 2006). Topography is flat to rolling country with the trial on flat ground. The dominant pasture species are perennial ryegrass (*Lolium perenne*) and white clover (*Trifolium repens*). The P x N study (Mackay *et al.* 2008) is made up of 5 rates of P fertiliser added to sustain Olsen P levels of 24, 30, 42, 49 and 76 (mg L^{-1}), and two rates of N (0 and 400 kg N ha^{-1} y^{-1}) applied as urea. Treatments are replicated 4 times in a randomized block design. The study area is excluded from grazing, with clippings removed at each harvest.

7.2.2. Data collection

Field visits were made to the Colyton site in October-2006, March-2007, June-2007 and September-2007. In each of the 40, 2m x 5m experimental plots pasture height was recorded using a rising plate pasture meter (FILIP^{TM}), pasture samples were cut at grazeable height for laboratory analysis for pasture N, P, K, Ca, Mg, Na and S, and reflectance factor spectra of the pasture swards were acquired *in situ* using an ASD FieldSpec$^{®}$ Pro FR spectroradiometer (ASD Inc., Boulder, CO, USA) attached to the Canopy Pasture Probe (CAPP) top-grip described in Chapter 2. A white ceramic tile was used as a reflectance standard (Sanches *et al.* 2009, Chapter 3).

7.2.3. Spectral data processing and analyses

Ten reflectance factor spectra were acquired from each plot and later averaged using the SpectraProc software (Hueni and Tuohy 2006) to form a unique spectrum per plot. Using The Unscrambler$^{®}$ 9.7 software (CAMO, Oslo, Norway), the spectra were then smoothed by applying a Savitzky-Golay filter (Savitzky and Golay 1964) with a window size of 81 and a polynomial order of 4, followed by the calculation of the first derivative of the reflectance factor using the Savitzky-Golay algorithm with window size of 3 and polynomial order of 2. Spectral data in the wavelength regions between 350-419 nm and 2401-2500 nm were eliminated because of the low signal-noise ratio in the instrument. All these procedures were applied to minimize noise and to enhance spectral features in the spectral data collected.

The first derivative reflectance (FDR) data between 420 and 2400 nm were used to build calibration models to predict pasture nutrients of the *added P* treatments and *added P+N* treatments using partial least squares regression (PLSR). The accuracy of the models was tested using the leave-one-out full cross-validation method. The optimal number of latent variables (principal components) used in the PLSR were determined by choosing the latent variables that produced the lowest root mean square error of cross-validation (RMSECV). Regression analyses were done for each of the two datasets (added P treatments = 20 samples by 4 harvests, added P and N = 20 samples by 4 harvests) and for the two datasets combined (160 samples). Regressions using the first derivative of the absorbance (Log 1/R) spectra rather than the FDR were also conducted, but the results were inferior (not shown). The accuracy of the PLSR calibration models were assessed in terms of coefficient of determination (R^2), root mean square error of cross-validation (RMSECV), slope, bias and the ratio of standard deviation (SD) of nutrient measured to the RMSECV (SD/RMSECV) for the validation dataset. Accurate prediction is shown by high R^2, low RMSECV, slope close to one, bias close to zero, and high SD/RMSECV. The RMSECVs for the calibrations of spectral data against nutrient concentration (% DM) and nutrient mass (kg ha^{-1}) cannot be directly compared because they have different units. In this case the SD/RMSECV ratio, which enables the evaluation of the RMSECV in terms of the SD of the measured data, was used to compare the prediction models for nutrient concentration and mass, the higher the value of this ratio the better.

To assess the importance of each wavelength for predicting plant P and N concentration and mass, the coefficients for the PLSR calibration models were examined. These plots summarise the relationship between the predictor variables and the pasture nutrients for the model selected. The higher the absolute regression coefficient values for a wavelength, the greater the importance of that particular wavelength in the PLSR calibration model. To assess which wavelengths were significant (useful and reliable) for the pasture nutrient prediction models, a Martens‟ Uncertainty Test, available in The Unscrambler® software, was used to estimate the approximate uncertainty variance of the PLSR coefficients under cross-validation. This test uses a Jack-knife method (Efron 1982), which was modified in order to compensate for rotational ambiguities of bilinear modelling, to estimate the uncertainty of the model parameters. During cross-validation, a number of sub-models are created with different data each time. For every sub-model, a set of model parameters (B-coefficients, scores, loadings and loading weights) is calculated. Variations over these sub-models are estimated so as to assess the stability of the results. For each variable the difference between the B-coefficient B_i in a sub-model and the B_{tot} for the total model is calculated. The Unscrambler® takes the sum of the squares of the differences in all sub-models to get an expression of the variance of the B_i estimate for a variable. With a *t*-test the significance of the estimate of B_i is calculated. Thus the resulting regression coefficients can be presented with uncertainty limits that correspond to 2 standard deviations under ideal conditions. Variables with uncertainty limits that do not cross the zero line are significant variables (Martens and Martens 2000, CAMO 2007).

7.2.4. Chemical analyses

Kjeldahl digests were conducted on the dried milled herbage. The N concentration of the Kjeldahl digests was determined using Flow Injection Analysis (FIA) Colorimetry with a modified Berthelot reaction (Searle 1984). Concentrations of P, K, Ca, Mg, Na and S were measured by digesting plant samples with nitric-perchloric acid followed by analysis using Inductively Coupled Plasma-Optical Emission Spectroscopy (ICP-OES, Boumans 1980). Nutrients concentrations were expressed as a percentage of dry matter (DM). To obtain nutrient mass per unit area, concentrations of N, P, K, Ca, Mg, Na and S (% DM) were multiplied by the mass (DM = 158 * plate meter height + 500) of dried pasture (kg DM ha^{-1}).

7.3. Results and Discussion

7.3.1. Phosphorus and Nitrogen

7.3.1.1. Effect of P and N fertilisers on grass-legume pasture composition

Increasing soil P status had no significant effect on sward composition (Table 7.1) or overall yield (Table 7.2). Added fertiliser N however reduced legume percentage in the sward (Figure 7.1). The average legume content of the *added P* treatment samples was 20%, whereas *added P+N* treatments samples averaged only 2% (Table 7.1). The average grass percentage was higher for the *added P+N* treatments (Table 7.1) as was the DM yield for *added P+N* treatments (Table 7.2), indicating that N limits grass growth even in well managed legume based pasture.

Table 7.1. Pasture botanical composition (percentages of grass, legume and weed) for each treatment.

	Treatment		Min	Max	Range	Mean	SD
Grass (%)	*Added P*						
	24*P*	(n = 16)	34	94	59	72^{b}	19
	30*P*	(n = 16)	46	97	51	82^{a}	14
	42*P*	(n = 16)	39	95	56	72^{b}	18
	49*P*	(n = 16)	17	91	73	67^{b}	23
	76*P*	(n = 16)	39	96	56	76^{ab}	19
	Total	(n = 80)	17	97	80	74	19
	Added P+N						
	24*P+N*	(n = 16)	72	100	28	93^{ab}	9
	30*P+N*	(n = 16)	88	100	12	95^{a}	4
	42*P+N*	(n = 16)	68	100	32	91^{b}	9
	49*P+N*	(n = 16)	86	100	14	95^{a}	5
	76*P+N*	(n = 16)	84	100	16	93^{ab}	6
	Total	(n = 80)	68	100	32	93	7
Legume (%)	*Added P*						
	24*P*	(n = 16)	4	54	51	18^{ab}	16
	30*P*	(n = 16)	1	52	51	13^{b}	13
	42*P*	(n = 16)	2	55	53	23^{a}	18
	49*P*	(n = 16)	8	79	72	26^{a}	19
	76*P*	(n = 16)	2	56	54	19^{ab}	17
	Total	(n = 80)	1	79	79	20	17
	Added P+N						
	24*P+N*	(n = 16)	0	7	7	1^{a}	2
	30*P+N*	(n = 16)	0	6	6	1^{a}	2
	42*P+N*	(n = 16)	0	7	7	1^{a}	2
	49*P+N*	(n = 16)	0	8	8	1^{a}	2
	76*P+N*	(n = 16)	0	15	15	4^{b}	4
	Total	(n = 80)	0	15	15	2	3
Weed (%)	*Added P*						
	24*P*	(n = 16)	0	35	35	11^{a}	10
	30*P*	(n = 16)	0	13	13	5^{b}	4
	42*P*	(n = 16)	0	16	16	5^{b}	5
	49*P*	(n = 16)	0	25	25	7^{ab}	8
	76*P*	(n = 16)	0	11	11	5^{b}	4
	Total	(n = 80)	0	35	35	7	7
	Added P+N						
	24*P+N*	(n = 16)	0	28	28	6^{ab}	8
	30*P+N*	(n = 16)	0	12	12	3^{b}	4
	42*P+N*	(n = 16)	0	25	25	8^{a}	8
	49*P+N*	(n = 16)	0	14	14	4^{b}	4
	76*P+N*	(n = 16)	0	14	14	4^{b}	4
	Total	(n = 80)	0	28	28	5	6

Mean values followed by different letters are different (*t*-test, $p < 0.05$).

Table 7.2. Pasture dry matter yield (kg ha^{-1}) for each treatment considering four cuts.

Treatment		Min	Max	Range	Mean	SD
Added P						
24*P*	(n = 16)	1669	2744	1074	2206^{ns}	310
30*P*	(n = 16)	1638	2665	1027	2252^{ns}	327
42*P*	(n = 16)	1685	2886	1201	2243^{ns}	352
49*P*	(n = 16)	1638	3075	1438	2349^{ns}	395
76*P*	(n = 16)	1559	2791	1232	2258^{ns}	358
Total	(n = 80)	1559	3075	1517	2262	344
Added P+N						
24*P+N*	(n = 16)	2222	3644	1422	2808^{ns}	367
30*P+N*	(n = 16)	2333	3597	1264	2858^{ns}	340
42*P+N*	(n = 16)	2349	3613	1264	2976^{ns}	359
49*P+N*	(n = 16)	2191	3439	1248	2881^{ns}	369
76*P+N*	(n = 16)	2380	3628	1248	2947^{ns}	357
Total	(n = 80)	2191	3644	1454	2894	355

^{ns} = not significant (t-test, $p < 0.05$).

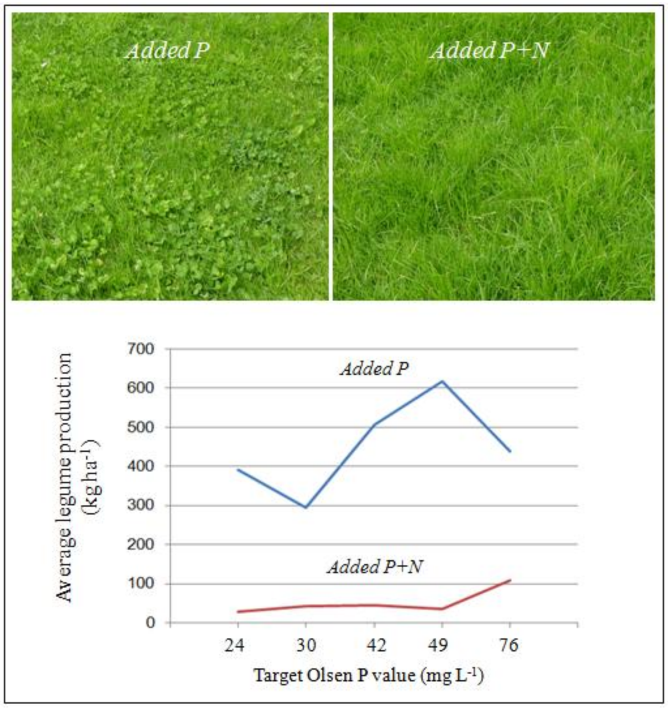

Figure 7.1. Photographs illustrating the legume composition of pastures receiving *added P* and *added P+N* (200kgN/ha). Averaged legume production (considering 4 cuts), which was calculated by multiplying the average legume % by the pasture mass, per treatment.

7.3.1.2. P and N concentration and mass

The concentration (%) and mass (kg ha^{-1}) of P and N in pasture dry mass are presented in Table 7.3. Mean herbage P concentrations were not affected by N application. Mean pasture N concentrations were higher (t-test, $p < 0.05$) in the *added P+N* samples. Mean P and N mass (kg ha^{-1}) from *added P+N* treatments were higher as consequence of the greater amount of DM from those treatments. The coefficients of variation (CVs) for concentration and mass of P were similar. The introduction of the N treatment (*added P+N)* increased the CV relative to the *added P* treatment. The CV is an important parameter to be considered because the lack of variation in sample populations can indicate that regression analysis may fail to establish any significant relationship for measured properties across individuals in that population. The CVs for N mass were higher than for concentration, with the CV slightly higher for *added P* samples compared to *added P+N* samples for N mass (Table 7.3).

Table 7.3. Summary statistics of N and P concentration (%) and mass (kg ha^{-1}) for *added P* and *added P+N* samples separately and combined.

Nutrient		Sample	n	Min.	Max.	Range	Mean	SD	CV
N	Concentration (%)	*added P*	80	2.99	4.53	1.54	3.73^{a}	0.37	0.10
		added P+N	80	3.33	4.93	1.60	3.98^{b}	0.35	0.09
		combined	160	2.99	4.93	1.94	3.85^{c}	0.38	0.10
	Mass (kg ha^{-1})	*added P*	80	53.91	130.40	76.49	84.80^{a}	18.65	0.22
		added P+N	80	83.46	150.84	67.38	114.97^{b}	15.78	0.14
		combined	160	53.91	150.84	96.93	99.88^{c}	22.92	0.23
P	Concentration (%)	*added P*	80	0.35	0.79	0.44	0.50^{ns}	0.08	0.15
		added P+N	80	0.31	0.68	0.37	0.48^{ns}	0.10	0.20
		combined	160	0.31	0.79	0.49	0.49^{ns}	0.09	0.18
	Mass (kg ha^{-1})	*added P*	80	6.47	15.93	9.46	11.21^{a}	1.70	0.15
		added P+N	80	8.02	20.77	12.75	13.92^{b}	2.86	0.21
		combined	160	6.47	20.77	14.30	12.56^{c}	2.71	0.22

Mean values followed by different letters are different (t-test, $p < 0.05$).
^{ns} = not significant (t-test, $p < 0.05$).

7.3.1.3. Pasture reflectance factor spectra

The reflectance factor spectra of the pasture samples collected (Figure 7.2a) were typical of the reflectance curve for green vegetation: low reflectance in the visible (Vis) part of the spectrum analysed (420-700 nm) due to strong absorption by leaf pigments, with a peak around 550 nm (green region); and high reflectance in the infrared (IR) over the range 700-1300 nm, which gradually decreases beyond 1300 nm due to water absorption (Knipling 1970). Visual interpretation showed the averaged reflectance factor spectrum of the *added P* treatment samples had higher values in the IR region, especially in the near-infrared (NIR) (700-1300 nm), compared to the spectrum of *added P+N* treatment samples. The spectral response of plants in NIR is dominated by leaf internal structure, which means the passage of light through leaves is influenced by factors such as the distribution of intercellular air spaces and the arrangement and size of cells (Kumar *et al.* 2001). Grasses are monocotyledonous and structurally different from dicotyledonous legumes; so pasture swards with and without legume species, or with different amounts of legume are likely to have different reflectances in the NIR, that need to be considered in any analysis. In fact, Verdebout *et al.* (1994) reported higher NIR reflectance for dicotyledonous compared to monocotyledonous plants due to the spongy nature of the leaf mesophyll of dicotyledonous compared to monocotyledonous, which is more compact. Where there are more air spaces and more air-water boundaries in leaves with spongy mesophyll, there is more scattering of radiation (translated into higher reflectance in the NIR). Leaves with a compact mesophyll have fewer air spaces, allowing more transmission and less scattering of radiation.

Reflectance has much larger magnitude in NIR than in Vis wavelengths. The *added P* treatment spectrum showed higher reflectance factor values than the *added P+N* treatment spectrum (Figure 7.2a) across the entire spectral range examined, and this difference was significant (*t*-test, $p < 0.05$). To better evaluate the spectral differences between *added P* and *added P+N* treatment spectra, the percentage difference in reflectance factor was calculated by subtracting the *added P+N* averaged spectrum from the *added P* averaged spectrum and then expressing the difference as a percentage of the reflectance factor of the added P spectrum, at each wavelength. For example, at 671 nm the reflectance factor difference is 0.003 (0.014 in *added P* averaged spectrum – 0.011 in *added P+N* averaged spectrum), which is equivalent to a 22% decrease in relation to the *added P* averaged spectrum; at 1108 nm the reflectance factor difference is 0.043 (0.519 in *added P* averaged spectrum – 0.476 in *added P+N* averaged spectrum), which is equivalent to an 8% decrease in relation to the *added P* averaged spectrum.

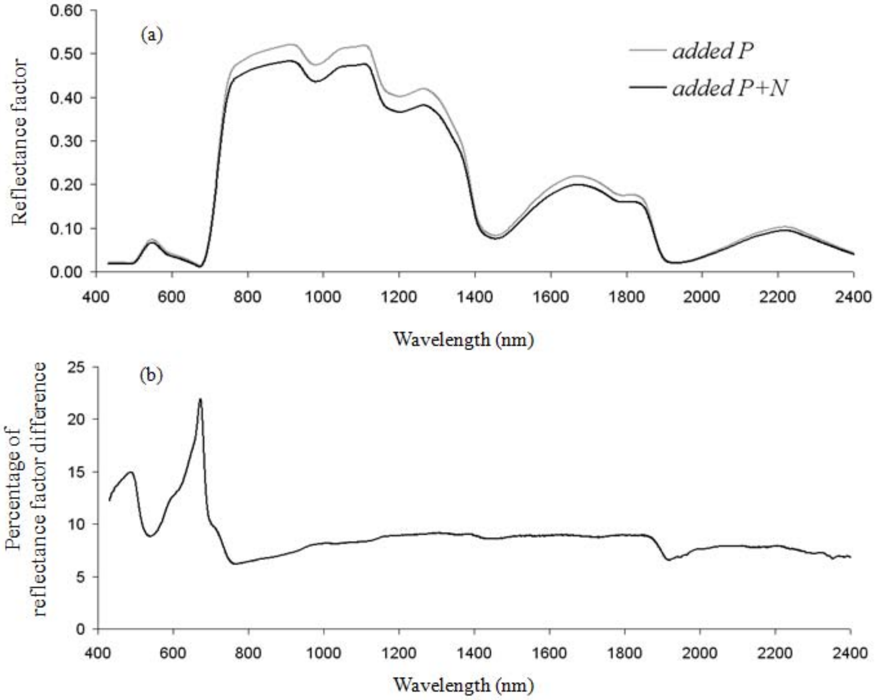

Figure 7.2. (a) Averaged reflectance factor spectra of *added P* and *added P+N* samples. (b) Percentage difference in reflectance factor, which corresponds to the percentage by which the reflectance factor of the *added P* averaged spectrum is higher than the *added P+N* averaged spectrum, at each wavelength.

With the difference spectra (Figure 7.2b), the greatest differences (over 10%) between the two spectra were observed in the Vis region, with the maximum peaks of difference at 471-496 nm (15%) and 671-675 nm (22%), blue and red wavelengths respectively. It is in the blue and red wavelengths that absorption bands caused by electron transitions in chlorophyll are found (Curran *et al.* 1989). Chlorophyll pigments absorb violet-blue and red light for photosynthesis (Kumar *et al.* 2001). When N is non-limiting, grasses have a higher photosynthetic rate than legumes (Ross *et al.* 1972), because the photosynthetic N use efficiency of grasses is better than legumes (Del-Pozo *et al.* 2000). As the *added P+N* treatment pasture samples had, on average, more N than *added P* pasture samples, the additional N available for the plants would allow higher rates of photosynthesis, resulting in more light being absorbed by chlorophyll pigments in the blue and red wavelengths. The sensing of plant canopies compared with single leaves is more complex because of a number of factors, particularly the canopy structure. Norman and Campbell (1989) described canopy structure as the amount of above-ground plant material and its organization, including the size, shape and orientation of leaves, stems, flowers and seed heads. Grass and clover canopies are distinctly different, with the former having long, mostly, erect-leaves allowing light penetration into the canopy. Clover leaves have a planophile arrangement, with leaves positioned at right angles to the sun and ground for much of the day (Harris 1994). Differences in canopy architecture affect sunlight penetration and consequently reflectance. Galvao *et al.* (2005) showed higher reflectance for planophile than erectophile sugarcane varieties. Madeira *et al.* (2001) also found erectophile rough bluegrass absorbed more radiation within the canopy, thereby lowering reflectance, than the planophile homogeneous bent grass. In our study, the presence of a higher percentage of legume in the *added P* treatment encouraged by P fertiliser addition only, resulted in a more horizontal/planophile canopy structure, and a higher reflectance than the *added P+N* treatments (Figures 7.2a, 7.2b).

The pattern of differences between the averaged reflectance factor spectra of *added P* and *added P+N* samples presented above was observed for each of the treatments (10*P* versus 10*P+N*, etc.) (not shown). When limiting the comparison of spectra to the *added P* samples, average reflectance factor spectra of 24*P* and 49*P* treatments were different (*t*-test, $p < 0.05$) between 674-676 nm (red region) and 745-795 nm (NIR); but no differences in botanical composition or DM yield were observed between those treatments. The average reflectance factor spectra of 30*P* and 49*P* treatments were different (*t*-test, $p < 0.05$) between 758-785 nm (NIR); and 30*P* treatment had a higher (*t*-test, $p < 0.05$) mean content of grass (82%) and lower mean content of legume (13%) compared to 49*P* treatment (mean values of 67% of grass and 26% of legume). Among the *added P+N* samples there were no differences ($p < 0.05$) in the spectra between treatments. Therefore, the variability detected in pasture spectra among pastures with different P levels was reduced with the application of N fertiliser and the associated decline in legume content.

7.3.1.4. Correlation between FDR and pasture P and N concentration and mass

Highest correlation coefficients (> 0.6) between FDR and P were observed at the Vis wavelengths for P concentration and in the NIR region for P mass (Figure 7.3). For N concentration and mass the highest coefficients were observed at both Vis and NIR wavelengths. This is consistent with observations made by Mutanga *et al.* (2004), who found highest correlation coefficients between FDR and tropical pasture N and P concentrations at both Vis and NIR under 1800 nm. Mutanga *et al.* (2004) found the best correlation at 521 nm and 566 nm for N, and 639 nm for P, which relate closely to the important wavelengths of 522 nm and 565 nm for N, and 634 nm for P in the present study. Mutanga *et al.* (2004) observed peaks at 521 nm, 1123 nm and 1297-1298 nm for both N and P concentration, while in our study peaks were at 511-513 nm for P and N concentration, 705-706 nm for P and N concentration, and 671-672 nm for P concentration and N mass.

Marked similarities were found when comparing the correlograms for *added P* samples with the correlograms of *added P+N* samples, especially for P concentration. This was not the case for N. For *added P+N* samples, wavelengths in the mid infrared (MIR) (1300-1400 nm) presented the highest correlations between N and the FDR. Overall the correlations were higher for *added P* samples than for *added P+N* samples, especially in the Vis region. The analysis of the correlograms shows the application of N fertiliser did not influence the correlation between pasture P and FDR. In contrast the correlation between pasture N and FDR was affected, indicating the prediction of pasture N with and without added N fertiliser will have different accuracies if not factored into the analysis. The differences are partly associated with the changing nature of the sward (Figure 7.2) and perhaps with the reduction in variance of the measured N mass values when fertiliser N is added (Table 7.3).

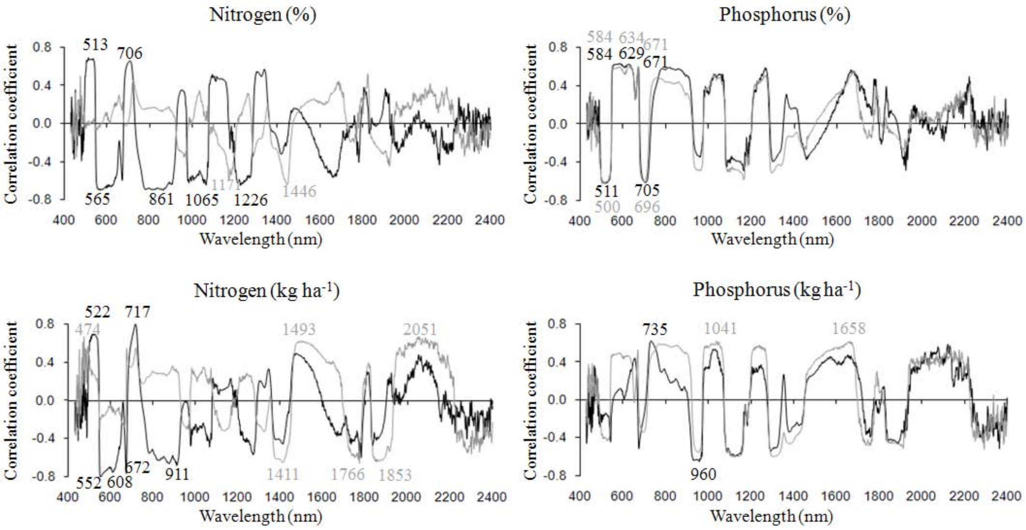

Figure 7.3. Correlation between FDR and pasture N and P concentration (%) and mass (kg ha^{-1}) for the *added P* (black lines) and *added P+N* (grey lines) samples. Wavelengths with high correlation coefficients are identified.

7.3.1.5. PLSR to predict pasture P and N concentration and mass

All PLSR based predictions for pasture P concentration and mass were inaccurate. However, some pasture N predictions were moderately accurate (Table 7.4, Figures 7.4 and 7.5).

Table 7.4. Summary of PLSR (cross-validation) results for predicting N and P concentration (%) and mass (kg ha^{-1}) of *added P* and *added P+N* samples analysed separately (each with n = 80) and combined (n = 160).

Nutrient		Sample	n	Latent variable	R^2	RMSECV	Slope	Bias	SD/RMSECV
N	Concentration (%)	*added P*	80	8	0.63	0.22	0.69	0.005	1.6
		added P+N	80	4	0.39	0.28	0.43	-0.002	1.3
		combined	160	4	0.22	0.34	0.26	0.000	1.1
	Mass (kg ha^{-1})	*added P*	80	6	0.78	8.75	0.81	-0.012	2.1
		added P+N	80	5	0.61	9.86	0.68	0.079	1.6
		combined	160	5	0.40	17.76	0.45	-0.024	1.3
P	Concentration (%)	*added P*	80	2	0.35	0.06	0.36	0.000	1.2
		added P+N	80	6	0.42	0.07	0.54	0.000	1.3
		combined	160	6	0.48	0.06	0.54	0.000	1.4
	Mass (kg ha^{-1})	*added P*	80	4	0.41	1.32	0.48	-0.007	1.3
		added P+N	80	1	0.31	2.40	0.32	0.001	1.2
		combined	160	5	0.30	2.28	0.36	0.000	1.2

The ability of the PLSR models to predict N and P concentration or N and P mass contrasted between elements. Coefficients of determination (R^2s) and slopes for pasture P prediction were low in all cases (R^2s < 0.42 and slopes < 0.54), improving slightly in prediction of P mass when analysing the *added P* treatment samples only, and for P concentration when analysing the *added P+N* dataset (Figure 7.4). The bias and SD/RMSECV ratio for P concentration were very similar to those for P mass (Table 7. 4).

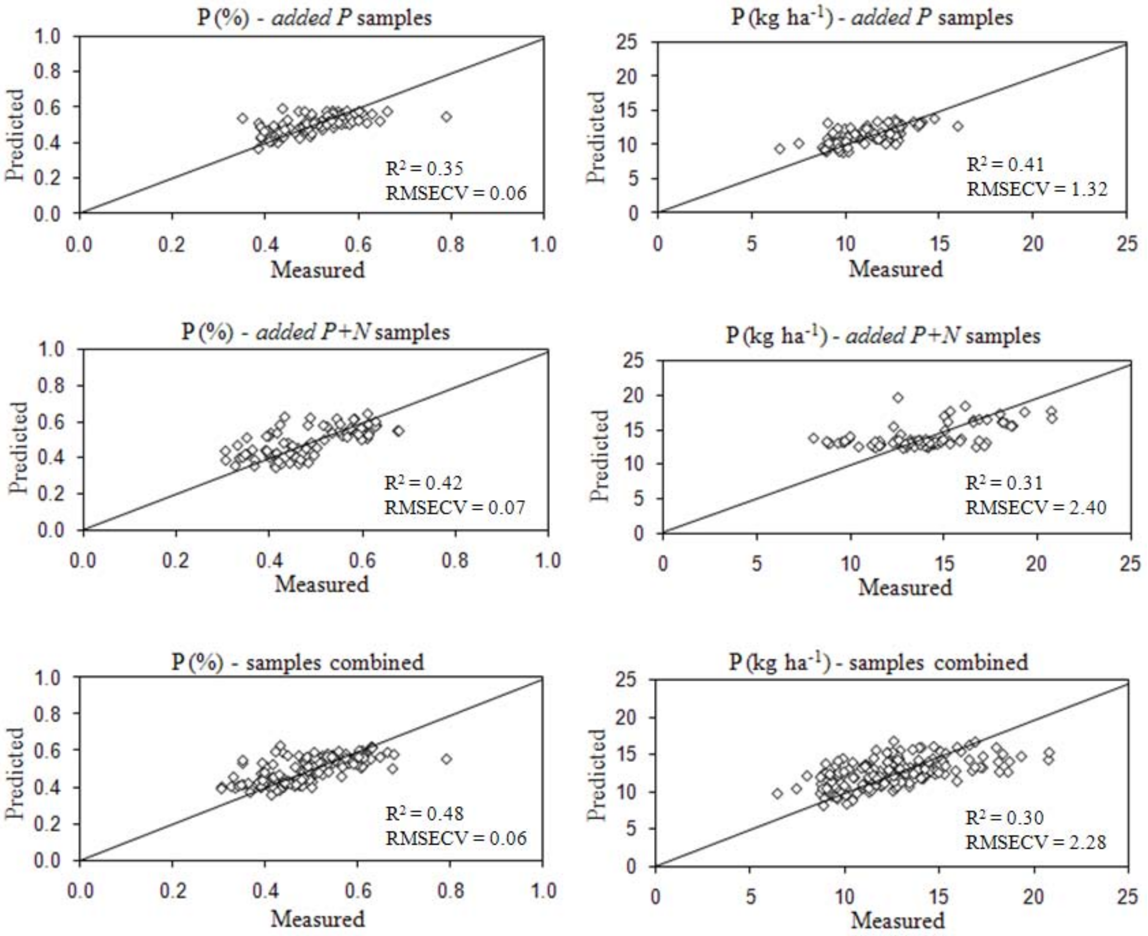

Figure 7.4. Relationship between measured pasture P concentration (%) and mass (kg ha^{-1}) and those predicted using PLSR cross-validation and FDR, of *added P* and *added P+N* samples separately (each with n = 80) and combined (n = 160).

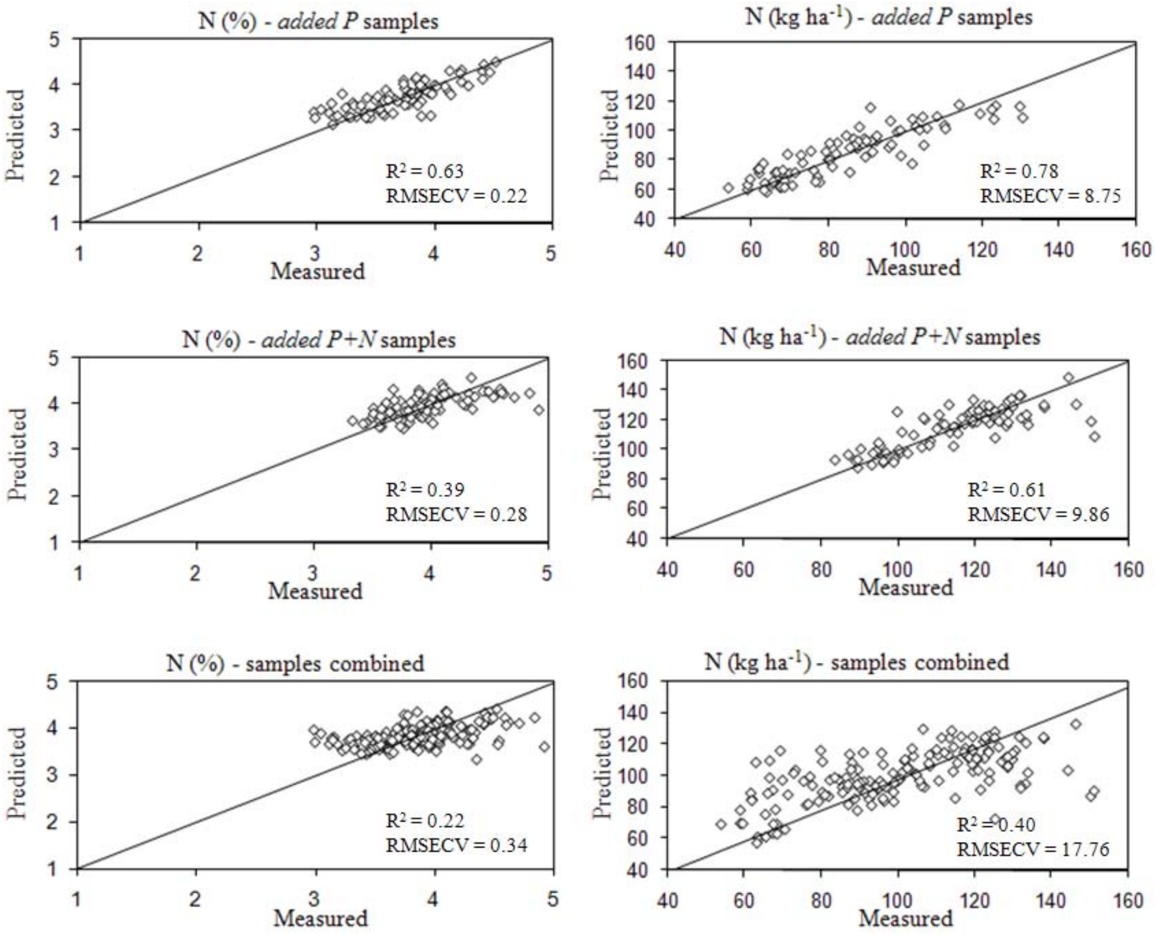

Figure 7.5. Relationship between measured pasture N concentration (%) and mass (kg ha^{-1}) and those predicted using PLSR cross-validation and FDR, of *added P* and *added P+N* samples separately (each with n = 80) and combined (n = 160).

For N prediction, better regression results were observed when analysing N mass rather than N concentration, and were independent of fertiliser N addition (Figure 7.5). The transformation of N concentration into mass increased the variation in this dataset contributing to an increase in the variance explained by the PLSR regression (R^{2}s). It is well known that the variation of samples in a dataset can impact on the predictive ability of the data, and this is the case here for pasture N estimation.

Comparing the regressions between samples sets, the results were similar when predicting pasture P. For the prediction of pasture N, the regressions for the *added P* samples were more accurate and precise, had higher R^{2}s, slope and SD/RMSECV and lower RMSECVs and bias than the regressions for the *added P+N* samples. Improved predictions of N in the *added P* samples can be linked to the fact that overall, the correlation of this nutrient with the FDR (Figure 7.3) was higher (higher values of correlation coefficients) in the *added P* samples than in the *added P+N* samples, at several wavelengths.

Regressions were also carried out using the combined datasets (Table 7.4, Figures 7.4 and 7.5) to check if a single model would be better than sub-models for *added P* and *added P+N* pastures. Estimation of P mass, N concentration and N mass were inferior in the combined model. Estimation of P concentration was slightly improved in the combined model, although the relationship was still poor ($R^2 < 0.50$). As mentioned before, the correlations between FDR and P concentration for plots with and without N fertiliser (Figure 7.3) were very similar, which explains why, when the samples were combined, the same correlation with the FDR was maintained. The greater range of samples had a positive influence on the regression results.

To examine if the 76*P* and 76*P+N* treatments, which had pasture P concentrations well above the optimum and into the luxury range (in excess of 0.65%), had an undue impact on the predictions of pasture P concentration or mass range, additional regressions were run (with the 76P data removed). Except for a small improvement in the prediction of pasture concentration with just the *added P* samples, all the new results had inferior accuracy compared to the analyses with all samples included. Also, no correlations were found between PLSR residuals and treatments in the calibration regressions developed, which means the highest errors when predicting pasture P and N were not associated with any specific treatment (e.g. N or P levels).

Mutanga *et al.* (2004) found R^2s for N% prediction of tropical pasture (0.60) were similar to the prediction of N concentration in *added P* samples and N mass in *added P+N* in this study. Their prediction of pasture P had a much higher R^2 (0.70) than ours (≤ 0.48). The RMSEP values of our N and P predictions are higher than the values presented by Mutanga *et al.* (2004). Compared with their study, where N content ranged from 0.38 to 2.00% (mean = 0.78%) and P ranged between 0.04-0.48% (mean = 0.18%); in this study nutrient concentrations were higher (Table 7.3), which explained the higher RMSEP values. Bogrekci and Lee (2005) using absorbance data and P concentration of 150 fresh Bahia grass samples (P range = 0.12-0.50%) collected in three sites in the Lake Okeechobee drainage basin in Florida, obtained a R^2 of 0.43 and RMSEP of 0.07%. This is similar to our result for predicting P concentration in *added P+N* samples (range = 0.31-0.68%).

Thulin *et al.* (2006) predicted *in situ* pasture crude protein (CP) of 75 samples collected from two sites in the south-eastern temperate zone of Victoria, Australia. Using derivative of absorbance data the prediction result was an R^2 of 0.62 and an RMSECV of 2.97% CP (equivalent to 0.48% N, since CP is 6.25 times the N content of forages). Their data were collected in 2000 and 2002 using different methods of capture. Limiting analysis to samples collected in 2002 (n = 45) improved the prediction accuracy (R^2 of 0.79 and a RMSECV of 2.39% CP (0.38% N)); this result was based on fewer samples compared to the present study, but had a much wider range of pasture N concentration (0.92-4.87% N) yielding a higher R^2 (similar R^2 if compared to the prediction of N mass for *added P* samples) and higher RMSE.

Kawamura *et al.* (2009a) using PLSR and FDR data have estimated New Zealand hill pasture N and P mass (kg ha^{-1}) with R^2 = 0.78 and RMSEP = 18.75, and R^2 = 0.94 and RMSEP = 2.57, respectively. Compared to our results, the prediction of N mass for *added P* samples had a similar R^2, but lower RMSEP. Our P predictions had much lower R^2s, although lower RMSEPs. In Kawamura *et al.* (2009a) the samples analysed had a wider range (N: 13.3-192.1 kg ha^{-1} and P: 1.6-36.9 kg ha^{-1}). Their results were improved when analysing CRDR data (R^2 = 0.89 and RMSEP = 17.14 for N, R^2 = 0.94 and RMSEP = 2.43 for P) instead of FDR.

The relationships between FDR and pasture P and N in the PLSR models selected (Table 7.4) are summarised in the regression coefficient plots (Figures 7.6 and 7.7). The wavelengths detected as important (significant) by the Martens" Uncertainty Test are highlighted in the plots. Some wavelengths which presented high values of regression coefficients were determined to be non-significant in the Martens" Uncertainty Test. These wavelengths were mainly localized on the margins of the spectrum and have higher noise (low signal-to-noise ratio). Important wavelengths for P and N prediction models were observed across the spectrum analysed, at Vis, NIR and MIR regions. The Martens" Uncertainty Test identified that only 2% of the original wavelengths (n = 1981) used in the regression to predict pasture P were retained in all 4 regressions (regressions predicting P concentration and mass in *added P* and *added P+N* samples, separately). These wavelengths were 1369-1374 nm, 1651-1660 nm, 1662-1668 nm, 1670-1673 nm, 1677-1683 nm and 1860-1863 nm. The same low percent of wavelengths were commonly selected as important in all 4 regressions predicting N, and were localized at 473-476 nm, 1034-1040 nm, 1108 nm, 1338 nm, 1344 nm, 1356-1363 nm, 1399 -1405 nm and 1416-1417 nm.

The low number of common wavelengths for the prediction of two pasture nutrients, highlights the importance of obtaining representative datasets that embrace all the biophysical factors (e.g. pasture type, canopy structure) likely to affect the relationship when building prediction calibrations. It is also important to be cautious when developing the calibrations on the choice of units to express nutrient. The present study highlights both these points showing that the practice of N fertiliser application to a legume based pasture and the simple conversion from nutrient concentration to mass impacts significantly on the pasture P and N prediction calibration. In the process of developing a calibration function, embracing as much of the variation in the field as possible is important; and in the promotion and use of that calibration function, one must ensure that any sampling falls within the limits of the datasets used in the construction of the regression.

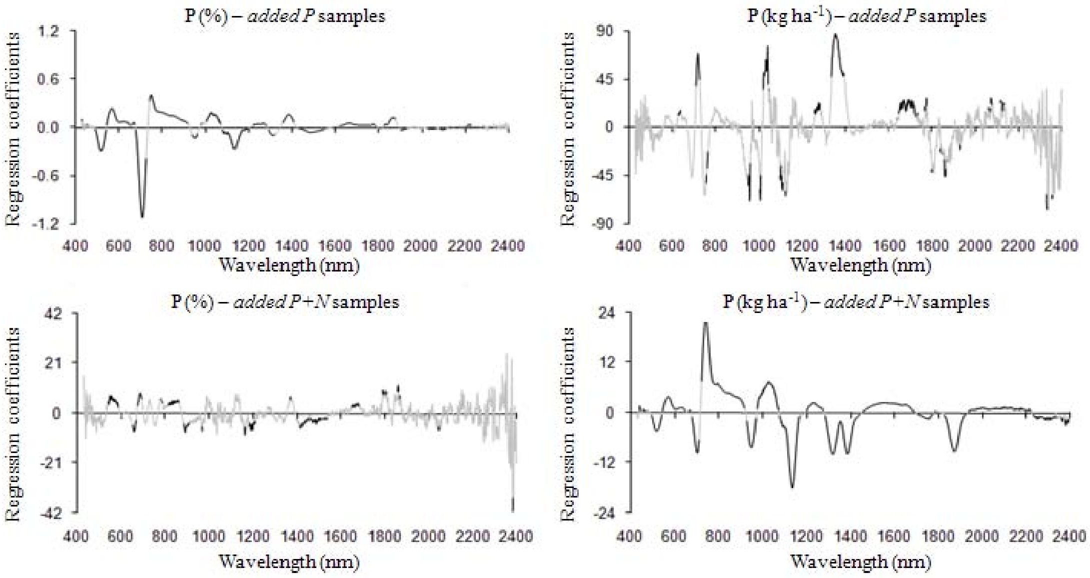

Figure 7.6. Regression coefficients of PLSR models between FDR and pasture P concentration (%) and mass (kg ha^{-1}) using the *added P* and *added P+N* samples analysed separately. Plotted in black are the significant wavelengths for the regression according to the Martens‟ Uncertainty Test.

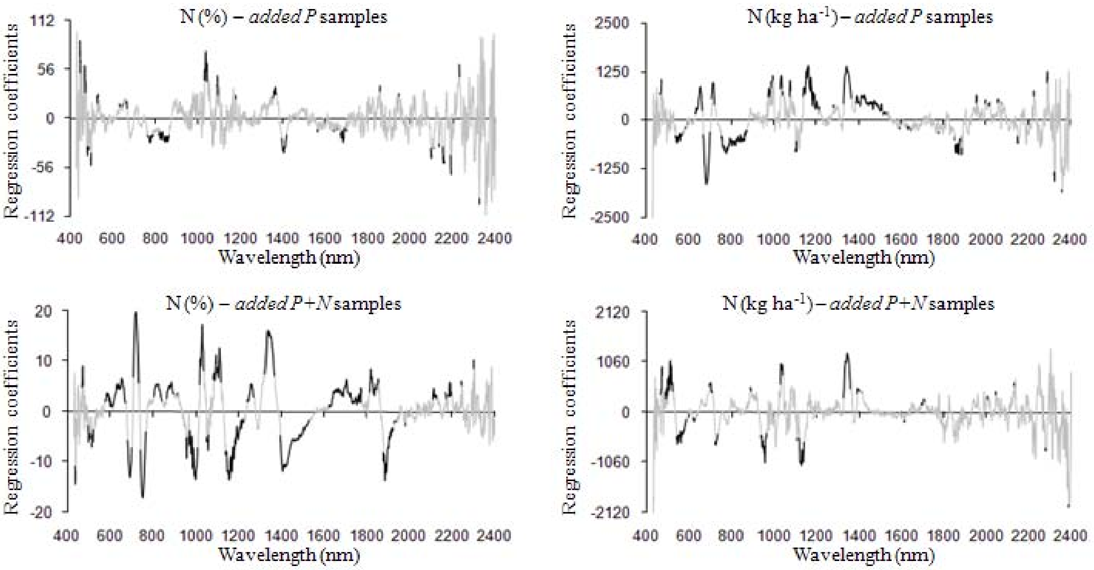

Figure 7.7. Regression coefficients of PLSR models between FDR and pasture N concentration (%) and mass (kg ha^{-1}) using the *added P* and *added P+N* samples analysed separately. Plotted in black are the significant wavelengths for the regression according to the Martens‟ Uncertainty Test.

7.3.2. Pasture potassium, calcium, magnesium, sodium and sulphur predictions

7.3.2.1. Pasture nutrient concentrations and masses

All 40 pasture plots receive applications of K, Ca, Mg, Na and S as a basal dressing twice a year to ensure they are not limiting pasture growth (Mackay *et al.* 2008). Comparing the nutrient concentration of the pasture plots with and without N fertiliser (Table 7.5), average K and Mg concentrations were similar. Mean Ca concentration values were higher for *added P* pasture samples (pasture with average legume content = 20%). In general, dicotyledonous plants have a higher cation exchange capacity, providing improved ability to compete with grasses for Ca (Laidlaw and Teuber 2001). Conversely, the *added P+N* samples (pastures with average legume content = 2%) had higher mean values of Na and S concentrations. Greater Na concentration in ryegrass than in white clover has been previously reported by Chiy and Phillips (1996). The higher concentration of S can be explained by the fact that grasses compete more strongly for S than legumes (Walker *et al.* 1956). For nutrient mass, the *added P+N* samples had higher mean values of all nutrients analysed; the result of greater DM production of those pastures.

Table 7.5. Summary of statistics of K, Ca, Mg, Na and S concentration (%) and mass (kg ha^{-1}) for *added P* and *added P+N* samples separately and combined.

Nutrient		Sample	Min	Max	Range	Mean	SD	CV
K	Concentration (%)	*added P*	1.51	3.86	2.35	2.84^{ns}	0.57	0.20
		added P+N	1.30	3.93	2.63	2.78^{ns}	0.69	0.25
		combined	1.30	3.93	2.63	2.81^{ns}	0.63	0.22
	Mass (kg ha^{-1})	*added P*	40.30	85.79	45.49	63.12^{a}	11.28	0.18
		added P+N	38.81	129.00	90.19	79.26^{b}	18.19	0.23
		combined	38.81	129.00	90.19	71.19^{c}	17.12	0.24
Ca	Concentration (%)	*added P*	0.42	1.14	0.72	0.67^{a}	0.17	0.26
		added P+N	0.44	0.85	0.41	0.62^{b}	0.10	0.16
		combined	0.42	1.14	0.72	0.65^{a}	0.14	0.22
	Mass (kg ha^{-1})	*added P*	8.63	32.32	23.69	15.45^{a}	5.62	0.36
		added P+N	10.58	29.13	18.55	17.96^{b}	4.28	0.24
		combined	8.63	32.32	23.69	16.70^{c}	5.14	0.31
Mg	Concentration (%)	*added P*	0.18	0.38	0.20	0.27^{ns}	0.05	0.19
		added P+N	0.15	0.43	0.28	0.29^{ns}	0.06	0.22
		combined	0.15	0.43	0.28	0.28^{ns}	0.06	0.21
	Mass (kg ha^{-1})	*added P*	3.32	10.39	7.07	6.27^{a}	1.89	0.30
		added P+N	4.23	14.86	10.63	8.47^{b}	2.49	0.29
		combined	3.32	14.86	11.54	7.37^{c}	2.46	0.33
Na	Concentration (%)	*added P*	0.10	0.65	0.55	0.31^{a}	0.15	0.49
		added P+N	0.15	1.24	1.09	0.55^{b}	0.26	0.47
		combined	0.10	1.24	1.13	0.43^{c}	0.24	0.57
	Mass (kg ha^{-1})	*added P*	1.67	17.40	15.73	7.35^{a}	4.35	0.59
		added P+N	3.45	42.31	38.86	16.32^{b}	8.78	0.54
		combined	1.67	42.31	10.64	11.83^{c}	8.24	0.70
S	Concentration (%)	*added P*	0.29	0.50	0.21	0.39^{a}	0.04	0.10
		added P+N	0.34	0.49	0.15	0.42^{b}	0.03	0.08
		combined	0.29	0.50	0.21	0.40^{c}	0.04	0.10
	Mass (kg ha^{-1})	*added P*	6.30	11.15	4.84	8.75^{a}	1.26	0.14
		added P+N	8.87	16.76	7.89	11.99^{b}	1.66	0.14
		combined	6.30	16.76	10.46	10.37^{c}	2.19	0.21

Mean values followed by different letters are different (*t*-test, $p < 0.05$).

^{ns} = not significant (*t*-test, $p < 0.05$).

Except for K, the CV for Ca, Mg, Na and S increased when the nutrients were transformed into mass. The CVs for those nutrients varied between 0.08-0.49 when analysed in concentration, and between 0.14-0.59 when analysed in mass.

7.3.2.2. Correlation with FDR

With the exception of S, acceptable correlations (R > 0.6) between FDR and the pasture cations were observed (Figure 7.8). Again except for S, the correlograms for concentration and mass were similar for the four cation nutrients, with marked similarity observed between the correlograms of *added P* and *added P+N* samples.

Some striking similarities were noticed between P and K correlograms (Figures 7.3 and 7.8, respectively) and for Ca, Mg and Na correlograms, reflecting in some cases high co-correlation between nutrients (Table 7.6). The correlation between Mg and Na in the pasture samples analysed varied from 0.82 (nutrient concentration in *added P* samples) to 0.91 (nutrient mass in *added P* samples), and between Ca and Mg from 0.59 (nutrient concentration in *added P* samples) to 0.88 (nutrient mass in *added P+N* samples). Mutanga *et al.* (2004) found that correlograms (nutrient concentration of tropical pasture with FDR) for P and K were similar to each other, while Ca and Mg correlograms were different. In Mutanga *et al.* (2004), the intercorrelations between P and K (0.74) and between Ca and Mg (0.60) were higher and lower, respectively, than in this study (Table 7.6).

Table 7.6. Intercorrelation of measured nutrient concentration and mass, for *added P* and *added P+N* samples.

	added P samples:								*added P+N* samples:						
Conc.	N	P	K	Ca	Mg	Na	S	Conc.	N	P	K	Ca	Mg	Na	S
N	1.00							N	1.00						
P	-0.39*	1.00						P	-0.03	1.00					
K	-0.44*	0.56*	1.00					K	0.30*	0.60*	1.00				
Ca	0.45*	-0.36*	-0.50*	1.00				Ca	-0.21	-0.31*	-0.62*	1.00			
Mg	0.55*	-0.44*	-0.69*	0.59*	1.00			Mg	-0.05	-0.48*	-0.74*	0.75*	1.00		
Na	0.55*	-0.44*	-0.68*	0.43*	0.82*	1.00		Na	-0.09	-0.51*	-0.84*	0.77*	0.85*	1.00	
S	-0.09	0.38*	0.20	-0.16	0.12	-0.01	1.00	S	-0.15	0.32*	0.31*	0.18	-0.08	-0.14	1.00
	added P samples:								*added P+N* samples						
Mass	N	P	K	Ca	Mg	Na	S	Mass	N	P	K	Ca	Mg	Na	S
N	1.00							N	1.00						
P	0.34*	1.00						P	0.26*	1.00					
K	0.10	0.49*	1.00					K	0.21	0.53*	1.00				
Ca	0.73*	0.15	-0.18	1.00				Ca	0.49*	0.10	-0.36*	1.00			
Mg	0.86*	0.27*	-0.13	0.80*	1.00			Mg	0.58*	0.02	-0.41*	0.88*	1.00		
Na	0.84*	0.25*	-0.14	0.70*	0.91*	1.00		Na	0.42*	-0.12	-0.63*	0.84*	0.87*	1.00	
S	0.68*	0.50*	0.14	0.45*	0.77*	0.73*	1.00	S	0.53*	0.43*	0.12	0.75*	0.67*	0.50*	1.00

Conc. – concentration; * Significant: $p < 0.05$

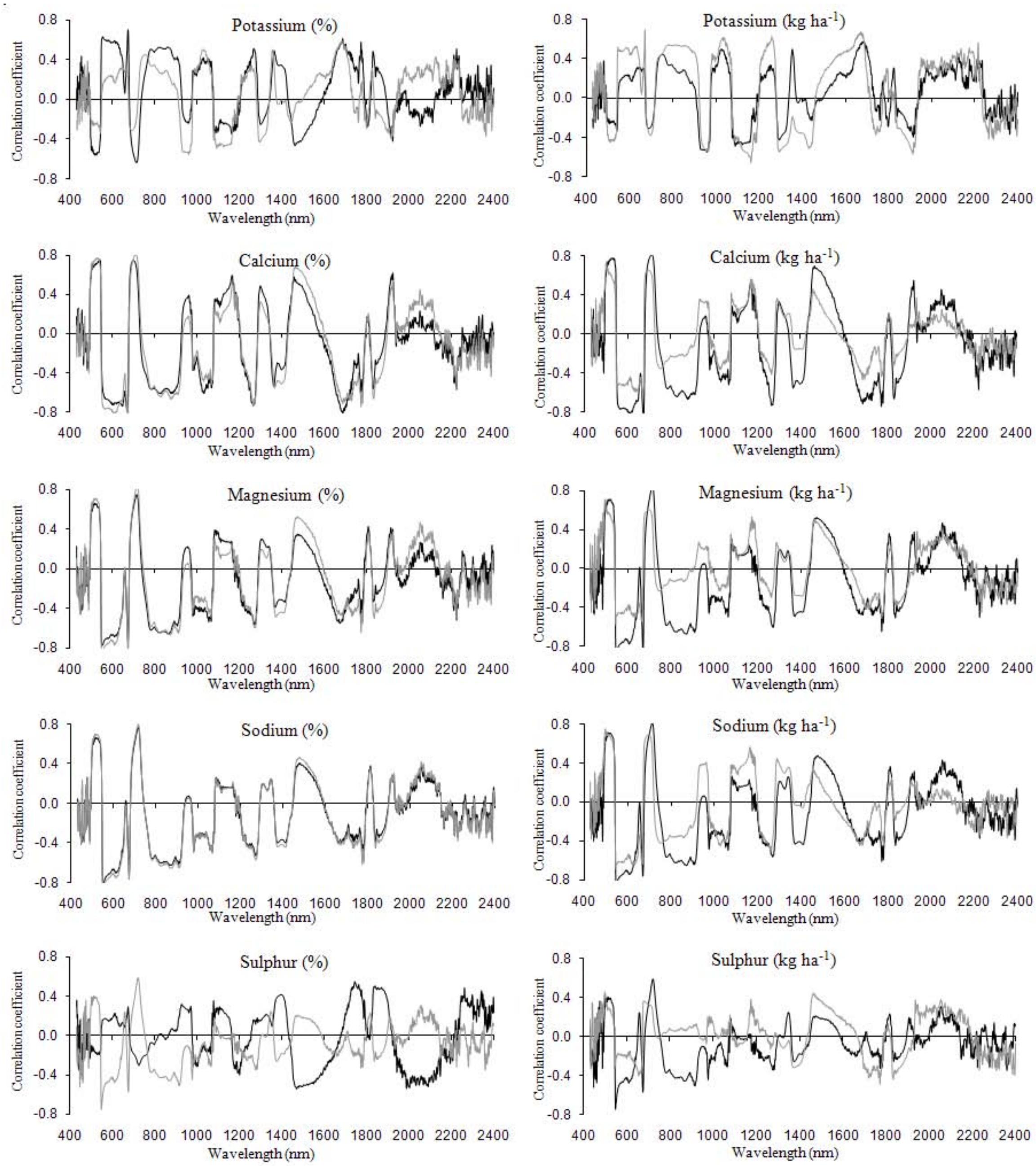

Figure 7.8. Correlation between FDR and pasture K, Ca, Mg, Na and S concentration (%) and mass (kg ha^{-1}) for the *added P* (black lines) and *added P+N* (grey lines) samples.

7.3.2.3. Prediction of pasture K, Ca, Mg, Na and S concentration and masses using PLSR models

Prediction accuracy varied greatly between the pasture K, Ca, Mg, Na and S analysed. Pasture S predictions were inaccurate, which was foreseen based on low correlations shown in the correlagrams between S and FDR (Figure 7.8). For K, Ca, Mg and Na predictions, accuracy varied depending on the data unit used (concentration or mass) and pasture treatments (*added P* or *added P+N*) included in the analysis. The R^2s, regression slopes and SD/RMSECV for Ca, Mg, Na and S predictions, but not K, were higher for nutrient mass than for concentration (Table 7.7), possibly reflecting the increased variation in these nutrients when expressed as mass rather than concentration.

The regression R^2s for prediction of pasture Ca and Mg in our study were higher than those of Mutanga *et al.* (2004), who reported $R^2 = 0.40$ and $R^2 = 0.52$ for tropical pasture Ca and Mg predictions, respectively, using continuum-removed derivative reflectance. These authors‟ R^2 for K prediction (0.60) was slightly higher than K concentration prediction in *added P* samples and lower than K concentration prediction in *added P+N* samples in the present study. The range in concentration of pasture Ca, Mg and K in the samples analysed in our study was wider than in the sample set analysed in Mutanga *et al.* (2004). Kawamura *et al.* (2009a) found New Zealand hill pasture K (kg ha^{-1}) estimations were $R^2 = 0.81$ and RMSEP = 28.41 using PLSR and FDR. In our study the R^2s of the pasture K predictions were inferior, and the RMSEPs lower. In Kawamura *et al.* (2009a) the samples analysed had a wider range of pasture K mass values (1.3-294.2 kg ha^{-1}).

Table 7.7. Summary of PLSR (cross-validation) results for predicting K, Ca, Mg, Na and S concentration (%) and mass (kg ha^{-1}) of *added P* and *added P+N* samples analysed separately (each with n = 80) and combined (n = 160).

Nutrient		Sample	n	Latent variable	R^2	RMSECV	Slope	Bias	SD/RMSECV
K	Concentration (%)	*added P*	80	5	0.57	0.37	0.63	0.000	1.5
		added P+N	80	6	0.68	0.39	0.72	0.002	1.8
		combined	160	6	0.58	0.41	0.63	0.002	1.5
	Mass (kg ha^{-1})	*added P*	80	5	0.41	8.71	0.49	-0.022	1.3
		added P+N	80	5	0.49	13.04	0.58	0.166	1.4
		combined	160	5	0.37	13.65	0.41	0.008	1.3
Ca	Concentration (%)	*added P*	80	5	0.66	0.10	0.71	0.001	1.7
		added P+N	80	4	0.46	0.07	0.53	0.002	1.4
		combined	160	4	0.61	0.09	0.63	0.000	1.6
	Mass (kg ha^{-1})	*added P*	80	6	0.80	2.54	0.83	0.055	2.2
		added P+N	80	6	0.62	2.66	0.68	0.050	1.6
		combined	160	6	0.66	3.01	0.70	0.002	1.7
Mg	Concentration (%)	*added P*	80	5	0.72	0.03	0.75	0.000	1.9
		added P+N	80	6	0.60	0.04	0.66	-0.001	1.6
		combined	160	6	0.62	0.04	0.66	0.000	1.6
	Mass (kg ha^{-1})	*added P*	80	6	0.87	0.69	0.88	0.001	2.7
		added P+N	80	6	0.70	1.37	0.73	0.009	1.8
		combined	160	7	0.65	1.47	0.69	0.000	1.7
Na	Concentration (%)	*added P*	80	4	0.61	0.10	0.65	0.000	1.6
		added P+N	80	6	0.66	0.15	0.71	0.001	1.7
		combined	160	7	0.66	0.14	0.69	-0.001	1.7
	Mass (kg ha^{-1})	*added P*	80	5	0.69	2.42	0.74	-0.013	1.8
		added P+N	80	6	0.68	5.00	0.73	0.106	1.8
		combined	160	7	0.66	4.83	0.69	-0.001	1.7
S	Concentration (%)	*added P*	80	4	0.23	0.04	0.31	0.000	1.1
		added P+N	80	1	0.00	0.03	0.01	0.000	1.0
		combined	160	4	0.23	0.03	0.27	0.000	1.1
	Mass (kg ha^{-1})	*added P*	80	5	0.49	0.90	0.58	-0.011	1.4
		added P+N	80	6	0.31	1.39	0.43	0.020	1.2
		combined	160	6	0.42	1.68	0.49	0.005	1.3

Comparing the regressions of *added P* samples with the regressions of *added P+N* samples, the highest R^{2}s were not always obtained for the samples with highest CVs, but that is not surprising since the differences between the two datasets reflect not only the variation of nutrients, but also variation due to other factors which influenced the pasture reflectance, including the botanical composition and standing pasture biomass. Based on the SD/RMSECV ratio, the prediction of Ca and Mg concentrations and masses were more accurate for the *added P* data than *added P+N* data; while the predictions for the other nutrients produced similar values of SD/RMSECV for both datasets. Better predictions of Ca and Mg for the *added P* samples, as with N predictions, can be linked to the fact that correlation of these nutrients with the FDR (Figure 7.8) was greater in the *added P* samples than in the *added P+N* samples, at several wavelengths. This result indicates that it is important to have samples that cover the complete range of nutrient contents that would be of interest when developing the calibration equation, but equally, that all other factors that are likely to influence reflectance and hence the relationship between nutrient content and reflectance are incorporated within the initial datasets. In general the predictions using large combined datasets are likely to have similar or inferior accuracy when compared to the regressions carried out from subsets of the data, as in the present study with the *added P* and *added P+N* subsets. This highlights the danger of collecting large datasets without an understanding of their influence and or importance in developing a robust calibration.

7.4. Conclusions

Field spectroscopy has the potential to predict pasture nutrient contents, but the quality of the calibrations and hence the prediction is dependent on pasture type, management practices such as fertiliser application, through to the unit of expression of nutrient status. The predictive power of the PLSR models developed in this study varied with each nutrient, with some pasture nutrient contents being predicted more successfully (e.g. N, Ca and Mg) than others (e.g. P and S). The expression of N, Ca, Mg, Na and S nutrient as mass (kg ha^{-1}) rather than nutrient as concentrations (percent of DM) resulted in a significant increase in the coefficient of variation of the samples which had a positive effect on the predictive ability of the PLSR models developed. The predictions of P and K did not benefit from the transformation of concentration into mass. It would be advantageous to have one universal model to predict multiple nutrients across a range of pasture types. The findings from this study indicate that a universal model may have less predictive accuracy. Specific calibrations for each nutrient and pasture type will increase the prediction accuracy, as was the case for the predictions of N, Ca and Mg on *added P* and *added P+N* pastures presented in this research. Improved confidence with which one can use a single model to predict multiple nutrients, or a model developed for individual nutrients, will only come through building a good understanding of the factors likely to influence the calibration function, rather than building larger datasets without this understanding. Without more research it is not advisable to extrapolate the PLSR models from this study to other sites.

CHAPTER 8:

Prediction of the nutrient concentration and mass of hill pasture using hyperspectral proximal sensing

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Some results of this study have been orally presented and published (full paper) in the proceedings of the 14th Australasian Remote Sensing and Photogrammetry Conference, Darwin, Australia, 29-03 October 2008.

Abstract

Hill pasture systems are much more complex than lowland areas, due to the high variability within very short distances caused by factors such as slope and aspect, resulting in diverse micro-climate and micro-topography, and the uneven nutrient transfer by livestock. The present study explored the use of hyperspectral proximal sensing to predict *in situ* nitrogen, phosphorus and potassium concentration (percent of dry matter) and mass (kg ha^{-1}) from a sheep grazed pasture of steep slope (> 20°), in the North Island of New Zealand. Partial least squares regressions between pasture nutrient concentration and the first derivative of reflectance (420-2400 nm), using a cross-validation method, yielded for N prediction: $R^2 = 0.47$, RMSECV = 0.40 and SD/RMSECV = 1.4; for P: $R^2 = 0.71$, RMSECV = 0.04 and SD/RMSECV = 1.9; and for K: $R^2 = 0.68$, RMSECV = 0.38 and SD/RMSECV = 1.8. The poorer results for N may be explained in part by the smaller sample size. Analyses carried out using N, P and K mass, were inferior. When the wavelengths were limited to those selected by the Martens" Uncertainty Test, the quality of pasture P% prediction increased slightly ($R^2 = 0.78$, RMSECV = 0.04 and SD/RMSECV = 2.0). It is concluded that there is a potential to use proximal sensing techniques to predict *in situ* nutrient contents of hill pasture, but this potential will realised only if a substantial dataset representing all the variability found in the field is evaluated.

Keywords: sheep pasture, hill country site, steep slope, hyperspectral data, nutrients.

8.1. Introduction

Hill country includes landscapes with slopes > 15°. Slope and aspect create a diverse micro-topography and micro-climate within very short distances. The livestock grazing and camping behaviours are influenced by slope and aspect; steeper slopes have increased nutrient loss, with the transfer of nutrients in excreta to tracks and low slope areas (Gillingham 1980). This heterogeneity and diversity makes the management of hill country pastures much more complex compared to lowland pastures (Harris 1994).

Low soil fertility along with low soil pH are major factors limiting pasture production on hill country (Edmeades *et al.* 1984). In New Zealand, low available soil nitrogen (N) concentrations are the primary factor limiting pasture production. The productivity of New Zealand hill country farms relies heavily on legume (clover) based pastures. Because of low clover content and yields, N fixation is often low in hill pastures. Fertilisers (mainly phosphorus (P) and sulphur (S)) are applied to stimulate the clover growth and N fixation. Fertilizer inputs represent the largest single input cost and therefore there is an increasing economic, as well environmental, pressure for more efficient use of fertiliser in hill country (Blennerhassett 2002).

To manage fertiliser nutrient inputs more efficiently, soil fertility and pasture nutrient content must be measured and monitored. Traditional methods of acquiring this kind of information are costly, especially when data from within hill country paddocks need to cover the diverse soil fertility status of hill pastures. One approach which has great potential for estimating pasture fertility at the within-paddock scale is the use of remotely sensed hyperspectral reflectance from the sward.

The reflectance (the ratio of radiation reflected from a surface to the radiation reaching that surface) spectra of plants in the visible (Vis), near infrared (NIR) and mid infrared (MIR) parts of the electromagnetic spectrum are dominated by plant pigments (chlorophyll, xanthophylls and carotenes), internal leaf structure (distribution of intercellular air spaces, arrangement and size of cells) and by leaf water content, respectively. Plant pigments strongly absorb energy in the Vis range, and the reflectance from leaves in those wavelengths is very low. In the NIR, the energy levels of light are not great enough for photochemical reaction so they are not absorbed by plant pigments, explaining the higher reflectance in those wavelengths. In MIR the reflectance is much lower compared to the NIR because of strong water absorption (Kumar *et al.* 2001).

The absorption bands of organic compounds of plant tissues noticed in the Vis (400-700 nm), NIR (700-1300 nm) and MIR (1300-2500 nm) regions are generally due to harmonics and overtones of the fundamental stretching frequencies of bonds (C-H, N-H and O-H) (Kumar *et al.* 2001). The organic chemical constituents of the plant tissue determine how much light is absorbed at a particular wavelength, thus reflectance spectra contain information on the chemical composition of that plant tissue (Foley *et al.* 1998). Under controlled laboratory conditions, foliar biochemicals of dried and ground samples have been successfully estimated by near infrared reflectance spectroscopy (NIRS) (Norris *et al.* 1976). For the analysis of fresh samples the task becomes complicated because the signature absorption bands for organic constituents can be masked by leaf water (Elvidge 1990). Moreover, if fresh plant samples are to be analysed *in situ*, the task of predicting organic constituents is even more challenging because of a number of factors such as the variation in leaf internal structure, atmospheric and background effects (Yoder and Pettigrew-Crosby 1995) and sward height (Lamb *et al.* 2002). Nevertheless promising results reported in the literature (Mutanga et al. 2004, Christensen *et al.* 2004, Sanches *et al.* 2006, Thulin *et al.* 2006, Kawamura *et al.* 2006) motivate further research into the development of proximal sensing for monitoring plant tissue composition *in situ*.

The present study investigates the prediction of *in situ* pasture N, P and potassium (K) concentrations and masses at a New Zealand hill country site using a portable spectroradiometer to acquire hyperspectral reflectance between 420 and 2400 nm.

8.2. Materials and methods

8.2.1. Study area

The study area was located on a sheep farm at Alfredton (around 40°38‘38” S 175°53‘52” E), in the North Island of New Zealand. The soil is classified as Brown Soil (Central Yellow-Brown Earths (Gibbs 1980) – Brown Soil (Hewitt 1993)). Data were collected from 36 sites (on north, south and east aspects; on slopes of 20 - 30°) set up for two experiments, one testing the effect of the application of different amounts of lime (24 plots: 12 facing north and 12 facing east), and another studying runoff (12 plots: 4 on north, 4 on south and 4 on east aspects).

Based upon temperature and rainfall, New Zealand pastures can be divided into six zones: northern, mid central, dry east coast, high-rainfall west coast, high country and cooler southern, but the boundaries of these zones are indistinct and each zone merges with the next. Alfredton is in the mid central zone, near the east coast zone. The mid central zone is characterized by few extremes of temperature and occasionally drought in summer. The hill pastures at this site are mostly composed of temperate grasses and legumes (cocksfoot, perennial ryegrass, dogstail and white and subterranean clovers, respectively) with very few subtropical or C4 grasses and some presence of less productive grasses, such as browntop and Yorkshire fog. In the dry east coast zone the summer drought is overwhelming and rainfall infiltration is limited by slope (Stewart and Charlton 2006). According to topography and elevation, New Zealand"s grasslands can be divided into three broad groups: high, hill and flat to rolling country (Valentine and Kemp 2007); Alfredton belongs to the hill country group, with slopes > 22º).

8.2.2. Data collection

Four field visits were made to Alfredton in August-2006 (winter), November-2006 (spring), February-2007 (summer), and May-2007 (autumn). The data were collected from swards under 1m x 0.5m exclusion cages. Data collection consisted of acquiring the spectral measurements and cutting of pasture samples at grazeable height. Reflectance factors of the pasture swards were acquired *in situ* using an ASD FieldSpec® Pro FR spectroradiometer (ASD Inc., Boulder, CO, USA) under artificial illumination provided by the Canopy Pasture Probe (CAPP) top-grip (Chapter 2). The reflectance standard was a white ceramic tile described by Sanches *et al.* 2009 (Chapter 3).

8.2.3. Spectral data processing and statistical analysis

At each plot (cage) ten spectra were acquired; these were later averaged using the SpectraProc software (Hueni and Tuohy 2006) to form a single spectrum per plot. These were then processed using The Unscrambler® 9.7 software (CAMO, Oslo, Norway). The spectral data were smoothed by applying a Savitzky-Golay filter (Savitzky and Golay 1964) with window size of 81 and polynomial order of 4, followed by calculation of the first derivative of the reflectance factor using the Savitzky-Golay algorithm with window size of 3 and polynomial order of 2. Spectral data in the wavelength regions between 350-419 nm and 2401-2500 nm were eliminated because of the low signal-to-noise ratio in the instrument. All these procedures were applied in an attempt to minimize as much noise as possible and to enhance spectral features in the spectral data collected.

The pre-processed first derivative reflectance (FDR) data between 420 and 2400 nm (1981 wavelengths) were then calibrated against the laboratory determined N, P or K content of the pasture samples using partial least squares regression (PLSR). The calibrations were tested using full cross-validation (leave-one-out method). The selection of the optimal number of latent variables (principal components) used in the PLSR was based on minimizing the additive prediction errors (minimum RMSECV). PLSR was carried out using the pasture nutrient content expressed as concentration (percentage of dry matter (DM)) or as mass (kg element ha^{-1}). PLSR calibration models using the reflectance factor, the absorbance (Log 1/R), and the first derivative of the absorbance spectra rather than the FDR were also conducted, but the results were inferior (not shown) to PLSR models using FDR.

The accuracy and precision of the PLSR calibration models were assessed in terms of coefficient of determination (R^2), root mean square error of cross-validation (RMSECV), slope, bias and the ratio of standard deviation of the nutrients measured to the RMSECV (SD/RMSECV) for the validation dataset. The SD/RMSECV ratio enables the evaluation of the RMSECV in terms of the SD of the measured data (N, P, and K concentration or mass measured by chemical analysis), and it is useful when comparing PLSR models of the different nutrients (nutrients with different ranges of samples) or the same nutrient in different units (concentration versus mass). Accurate and precise prediction is shown by high R^2, low RMSECV, slope close to one, bias close to zero, and high SD/RMSECV.

To assess the importance of each wavelength for the nutrient predictions, the PLSR coefficients plots were examined. These plots summarise the relationship between the predictor variables and the pasture nutrient concentration and mass for the models selected; the greater the absolute regression coefficient value of a wavelength, the greater the importance of that particular wavelength for that prediction model. To assess which wavelengths were significant (useful and reliable) for the pasture nutrient predictions models, a Martens" Uncertainty Test, available in The Unscrambler® software, was used to estimate the approximate uncertainty variance of the PLSR coefficients under cross-validation. This test uses a Jack-knife method (Efron 1982), which was modified in order to compensate for rotational ambiguities of bilinear modelling, to estimate the uncertainty of the model parameters. Under cross-validation, the number of sub-models created is one less that the total number of observations as the procedure iteratively leaves one observation out to compare predicted and observed values. For every sub-model, a set of model parameters (B-coefficients, scores, loadings and loading weights) is calculated. Variations over these sub-models are estimated so as to assess the stability of the results. For each variable the difference between the B-coefficient B_i in a sub-model and the B_{tot} for the total model is calculated. The Unscrambler® takes the sum of the squares of the differences in all sub-models to produce an expression of the variance of the B_i estimate for a variable. With a *t*-test the significance of the estimate of B_i is calculated. Thus the resulting regression coefficients can be presented with uncertainty limits that correspond to 2 standard deviations under ideal conditions. Variables with uncertainty limits that do not cross the zero line are significant variables (Martens and Martens 2000, CAMO 2007).

8.2.4. Nitrogen, phosphorus and potassium chemical analyses

From the 144 pasture samples collected in this study (36 plots at four dates), 4 samples could not be analysed (samples were too small), 48 samples were analysed for N using a wet chemistry method (samples from the runoff trial); 92 samples were analysed for

K (samples from the lime trial) and 140 samples were analysed for P (samples from both trials).

Pasture samples were dried in a forced air oven at 60°C for 48 hours, and weighed to determine pasture mass (kg DM ha^{-1}). The N concentration was determined by the Kjeldahl digest method. Concentrations of P and K were measured by a nitric-perchloric digest followed by analysis using Inductively Coupled Plasma-Optical Emission Spectroscopy (ICP-OES). The nutrient concentration was expressed as a percentage of DM. To obtain nutrient mass in kg ha^{-1} the concentrations of N, P and K (% DM) were multiplied by pasture mass (kg DM ha^{-1}).

8.3. Results and Discussion

8.3.1. Pasture spectra

The averaged reflectance factor spectra for each aspect were noticeably different (Figure 8.1a). The differences among spectra in certain waveband regions were statistically significant. Statistically significant differences (t-test, $p < 0.05$) were observed between north and south aspects for the NIR (between 720-1220 nm); and between north and east aspects for the Vis (between 420-511 nm and 585-698 nm), NIR (between 724-874 nm, 942-1017 nm and 1136-1210 nm) and MIR (between 1440-1478 nm, 1875-2083 nm and 2365-2400 nm) spectral regions. There was no significant difference between spectra collected from east and south aspects (Figure 8.1b).

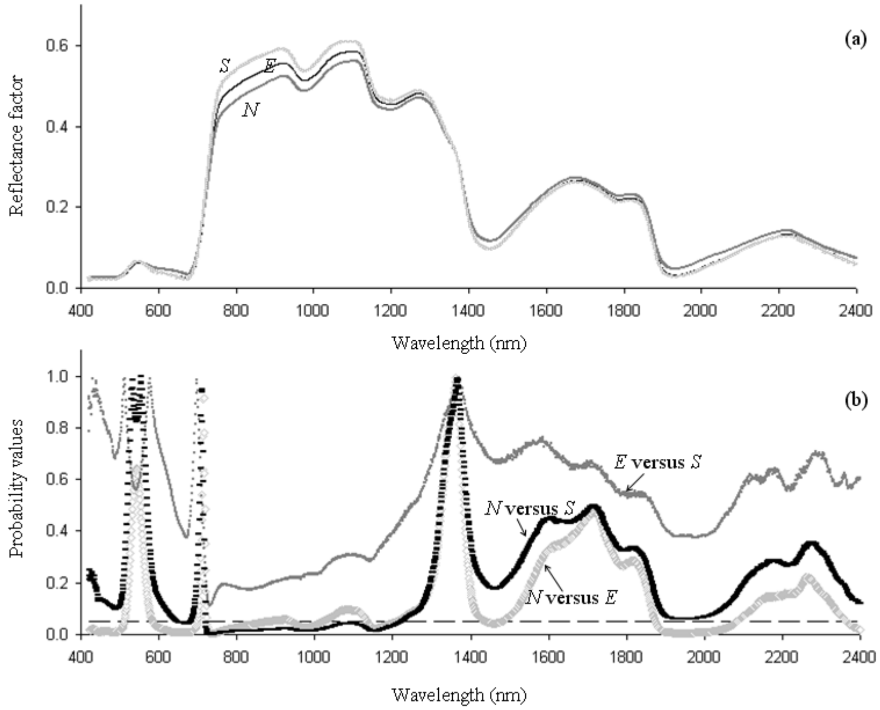

Figure 8.1. (a) Averaged reflectance factor spectra for each aspect (N – north, S – south, E – east). And (b) results of the t-test considering three aspects: N versus S, N versus E and E versus S. The dots plotted show the probability value, for each wavelength, when testing if the average reflectance factor spectra between classes are significantly different. Horizontal line in dashed black shows 95% ($p < 0.05$) confidence limit.

The wavelengths at which the averaged reflectance factor spectrum of north-facing slopes differed statistically from spectra of east and south-facing slopes in this study had been reported (Thenkabail *et al.* 2004) as the hyperspectral bands best correlated with several plant variables such as biomass (centred at 655 nm, 885 nm, 915 nm, 1085 nm, 1135 nm), soil background (centred at 495 nm, 655 nm), and plant moisture (centred at 980 nm, 1215 nm, 1445 nm, 2005 nm, 2035 nm).

In hill pastures, aspect is one parameter which influences the temperature and soil moisture, causing large differences in pasture composition and production between aspects. Average DM yield of north-facing slopes was much lower than on east and south-facing aspects (Table 8.1). The average DM yield collected from eastern and southern aspects was not statistically different (t-test, $p < 0.05$), but was significantly different from yield on the northern aspect. Sunny or north-facing aspects have higher soil temperatures than shady or south-facing aspects. Exposed to north-west winds and receiving greater levels of radiation, they are also much drier. Shady south facing slopes had less bare soil, higher plant densities and greater species diversity than sunny north facing slopes, which agrees with other observations in hill country (White 1990).

Table 8.1. Basic statistics of pasture DM yield (kg ha^{-1}) by aspect. DM yield values based on four cuts (August-2006, November-2006, February-2007 and May-2007).

Aspect	Min.	Max.	Mean	SD
North (n = 60)	7	2977	960	654
East (n = 64)	107	3035	1408	788
South (n = 16)	290	2462	1642	648

8.3.2. Pasture nutrient concentration and mass

The N, P and K concentrations and masses in the samples analysed (Table 8.2) are typical of low to moderate fertility New Zealand hill pastures.

Table 8.2. Basic statistics of nitrogen, phosphorus and potassium concentration (%) and mass (kg ha^{-1}) of the pasture samples analysed.

			Min.	Max.	Range	Mean	SD	CV
Nutrient concentration (%)	N (n = 48)		1.16	3.80	2.64	2.49	0.54	0.22
		North (n = 16)	1.69	3.29	1.59	2.45	0.48	0.20
		East (n = 16)	1.42	3.44	2.02	2.50	0.57	0.23
		South (n = 16)	1.16	3.80	2.64	2.53	0.60	0.24
	P (n = 140)		0.15	0.49	0.34	0.32	0.08	0.25
		North (n = 60)	0.17	0.49	0.32	0.30	0.09	0.30
		East (n = 64)	0.15	0.45	0.30	0.34	0.07	0.20
		South (n = 16)	0.22	0.49	0.28	0.37	0.07	0.18
	K (n = 92)		0.90	3.80	2.90	2.15	0.66	0.31
		North (n = 44)	0.90	3.40	2.50	1.97	0.73	0.37
		East (n = 48)	1.40	3.80	2.40	2.32	0.54	0.23
			Min.	Max.	Range	Mean	SD	CV
Nutrient mass (kg ha^{-1})	N (n = 48)		4.14	75.29	71.15	37.04	17.10	0.46
		North (n = 16)	11.6	67.63	56.03	36.36	15.64	0.43
		East (n = 16)	4.14	63.99	59.85	34.93	18.16	0.52
		South (n = 16)	8.61	75.29	66.68	39.83	18.13	0.46
	P (n = 140)		0.13	9.76	9.64	4.03	2.56	0.64
		North (n = 60)	0.13	7.61	7.48	2.94	2.10	0.71
		East (n = 64)	0.41	9.48	9.08	4.60	2.59	0.56
		South (n = 16)	1.11	9.76	8.65	5.81	2.43	0.42
	K (n = 92)		0.76	75.87	75.11	24.67	19.23	0.78
		North (n = 44)	0.76	53.28	52.52	16.52	14.33	0.87
		East (n = 48)	2.68	75.87	73.20	32.14	20.22	0.63

No significant differences (*t*-test, $p < 0.05$) were observed between average pasture N, P and K concentrations and masses from eastern and southern aspects; the northern aspect had significantly lower average P and K concentration and mass.

8.3.3. Correlation between FDR and pasture nutrients

Acceptable correlations ($R > 0.50$) were observed between the FDR over the range 420-2400 nm and pasture nutrient concentration and mass (Figure 8.2). This finding contrasts to that of Mutanga *et al.* (2004) who found very weak correlations between FDR and the N, P and K concentration of tropical pasture between 2000-2500 nm. Both studies find some similarities among N, P and K correlograms, and an inter-correlation between these nutrients. In the Mutanga *et al.* (2004) study, N, P and K correlograms had similar highly correlated peaks at blue (521 nm), NIR (721-747 nm, 1123, 1289-1297 nm) and MIR (1523-1531 nm) wavelengths; and R values between N-P = 0.41, N-K = 0.72 and P-K = 0.74. In the present study, the highest correlation coefficients for N, P and K concentration (%) had common wavelengths at blue (around 490 nm) and red (around 670 nm) regions. For P and K mass (kg ha^{-1}) correlograms were almost identical; green (around 560 nm) and red (around 630 nm) wavelengths had the highest correlation coefficient values. Overall correlation coefficients were higher for pasture P and K nutrients compared to N and for nutrient concentration (%) compared to nutrient mass (kg ha^{-1}). The correlations were: N-P (%) = 0.78; N-P (kg ha^{-1}) = 0.95; P-K (%) = 0.87; and P-K (kg ha^{-1}) = 0.98. The correlation between N and K could not be explored because there were no pasture samples which were analysed for both nutrients.

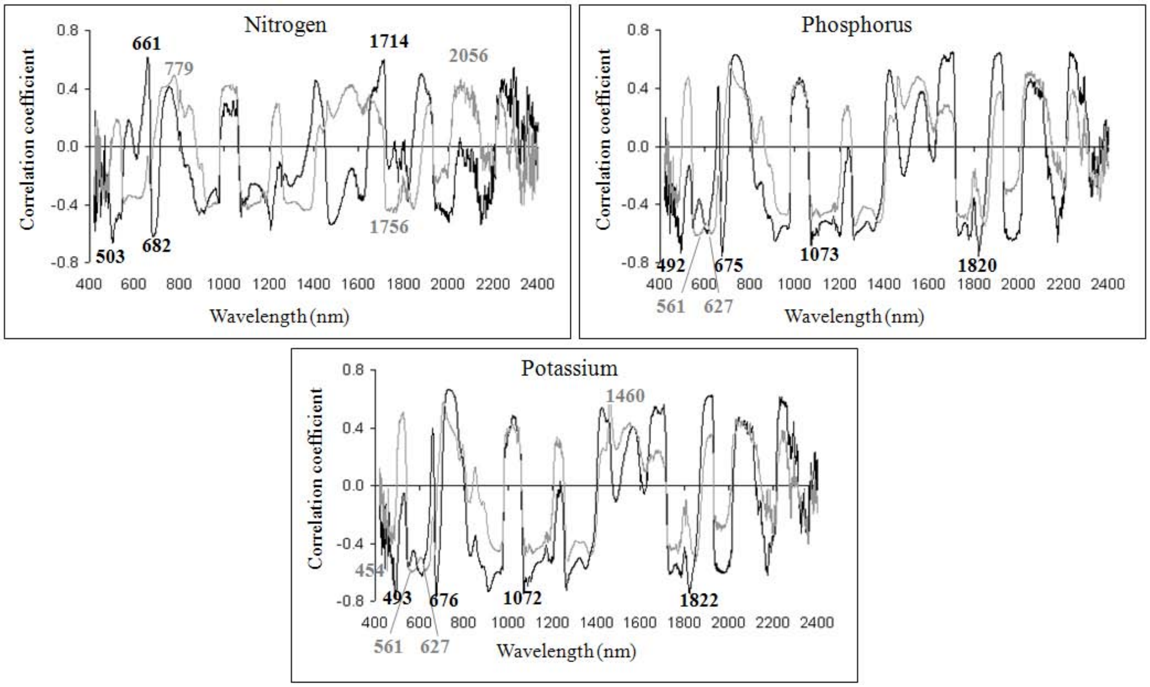

Figure 8.2. Correlation between FDR and pasture nitrogen, phosphorus and potassium concentration (%) and mass (kg ha^{-1}) represented in the plots in black lines and grey lines, respectively. Highest correlated wavelengths are identified.

8.3.4. Prediction of pasture nutrients

The PLSR models predicting pasture N, P and K concentrations (%) from FDR data yielded acceptable R^{2}s (> 0.5) and slopes for K and P predictions, but poor R^2 (< 0.5) and slopes for N prediction; with a small bias for all the predictions (Table 8.3 and Figure 8.3). The RMSECV obtained for N, P and K predictions was 0.40, 0.04 and 0.38, respectively, with the best SD/RMSECV ratio (lowest value) obtained for P and the worst for N.

Table 8.3. Summary of results for the PLSR models developed using the FDR data to predict pasture nitrogen, phosphorus and potassium concentrations (%) and mass (kg ha^{-1}). Model accuracy was tested internally using leave one out cross-validation. Results are presented for the analysis using all wavelengths between 420-2400 nm (1981 wavelengths) and when analysis was limited to the wavelengths selected by the Martens‟ Uncertainty Test.

Nutrient		Number of wavelengths	Latent variable	R^2	RMSECV	Slope	Bias	SD/RMSECV
Nitrogen (n = 48)	(%)	1981 (100%)	4	0.47	0.40	0.54	-0.011	1.4
	(kg ha^{-1})	1981 (100%)	1	0.14	16.05	0.14	-0.069	1.1
	(%)	71 (4%)	3	0.44	0.41	0.47	-0.005	1.3
	(kg ha^{-1})	1073 (54%)	1	0.14	16.05	0.14	-0.065	1.1
Phosphorus (n = 140)	(%)	1981 (100%)	7	0.71	0.04	0.75	-0.001	1.9
	(kg ha^{-1})	1981 (100%)	5	0.42	1.96	0.47	-0.003	1.3
	(%)	578 (29%)	7	0.78	0.04	0.80	0.000	2.0
	(kg ha^{-1})	502 (25%)	7	0.46	1.89	0.52	-0.001	1.4
Potassium (n = 92)	(%)	1981 (100%)	6	0.68	0.38	0.72	0.005	1.8
	(kg ha^{-1})	1981 (100%)	4	0.49	13.84	0.54	0.166	1.4
	(%)	115 (6%)	5	0.67	0.38	0.69	0.000	1.7
	(kg ha^{-1})	16 (1%)	2	0.36	15.50	0.36	-0.017	1.2

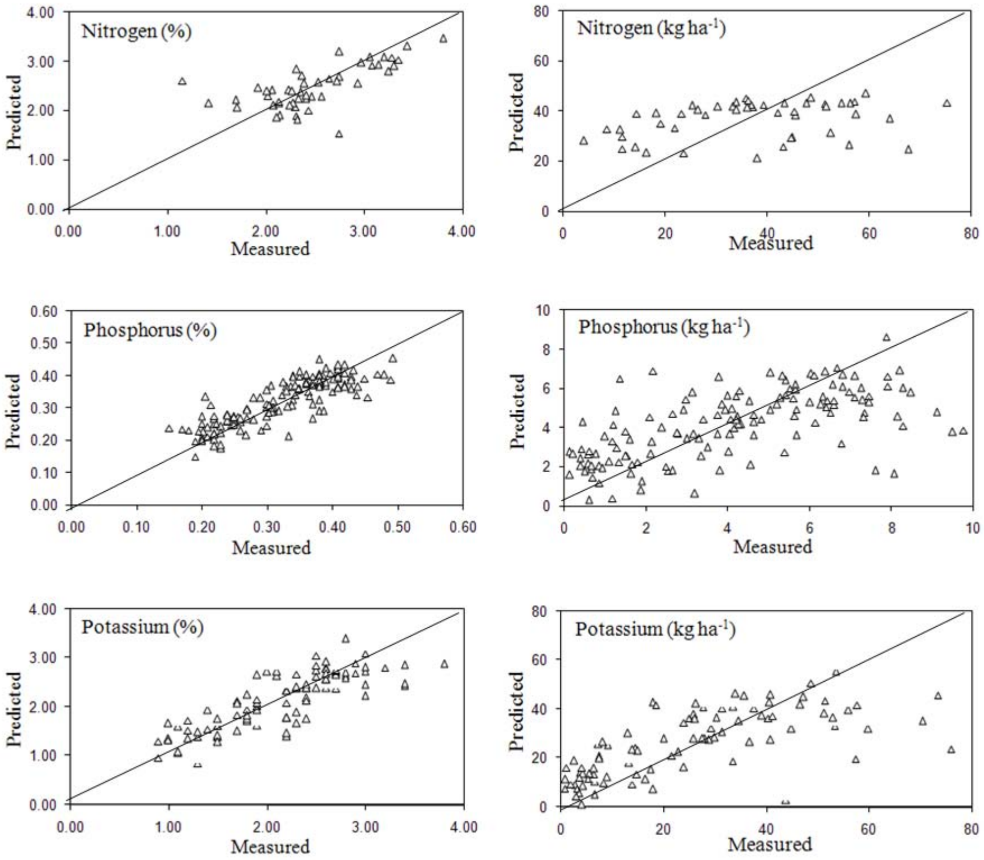

Figure 8.3. Relationship between measured and predicted pasture nitrogen, phosphorus and potassium concentration (%) and mass (kg ha^{-1}) using PLSR cross-validation method.

The RMSEP values for the predictions in the present study are much higher than the values presented by Mutanga *et al.* (2004) especially for pasture N and K concentrations. Compared with the study of Mutanga *et al.* (2004), where the N content ranged from 0.38 to 2.00% (mean = 0.78%), P ranged between 0.04-0.48% (mean = 0.18%), and K from 0.21 to 2.71% (mean = 0.96); in this study nutrient concentrations were higher and did not cover such a large range (Table 8.2). The percentage variation in pasture P concentration explained by the predictive model developed in this study and that of Mutanga *et al.* (2004) were similar ($R^2 = 0.71 – 0.70$). Bogrekci and Lee (2005) using absorbance data and P concentrations of 150 fresh Bahia grass samples collected at three sites in the Lake Okeechobee drainage basin in Florida, obtained an R^2 of 0.43 and RMSEP of 0.07%. That predictive model has lower accuracy than the model developed in this Chapter, despite having used a similar number of samples and range of P% (0.12-0.50%).

Thulin *et al.* (2006) predicted *in situ* pasture crude protein (CP) concentrations for 75 samples collected from two sites in the south-eastern temperate zone of Victoria, Australia. The predictive model using the derivative of the absorbance data had an R^2 of 0.62 and a RMSECV of 2.97% CP (equivalent to 0.48% N, since CP is 6.25 times the N content of forages). Their data were collected in 2000 and 2002, using different methods of capture. Limiting analysis to samples collected in 2002 ($n = 45$) improved the relationship (R^2 of 0.79 and a RMSECV of 2.39% CP (0.38% N)). This last result analysed a similar number of samples to our study but had a much wider range of pasture N concentration (0.92-4.87% N) yielding a similar RMSE, but much higher R^2.

Since low levels and narrow ranges in reference nutrient concentrations can cause regression model R^2 values to be misleading (Roberts *et al.* 2004), the ranges of the three nutrients analysed in this study were increased by transforming the nutrient concentration (%) into the amount of nutrient in the standing sward (kg ha^{-1}) expressed here as mass (Table 8.2). The idea to use the nutrients expressed as kg ha^{-1} was based on Yoder and Pettigrew-Crosby (1995), Jacquemoud *et al.* (1996) and Kawamura *et al.* (2009a). These authors reported an increase in biochemical variation when the reference data were expressed as amount (g cm^{-2}, kg ha^{-1}) instead of concentration (percent of dry weight). Indeed, in this study the variation of samples was increased when the nutrients were transformed into kg ha^{-1}; the coefficient of variation (CV), calculated by dividing the SD by the mean, varied between 0.22-0.31 for nutrient concentration (%), and between 0.46-0.78 for nutrient mass (kg ha^{-1}) (Table 8.2). When the regression models were calibrated against the mass (kg ha^{-1}), the predictions of the three nutrients decreased considerably in accuracy with respect to the R^2s, slopes and SD/RMSECV, despite the number of latent variables in the PLSR models having been reduced (Table 8.3 and Figure 8.3). In PLSR the latent variables represent linear combinations of the predictor variables chosen to describe as much of the variation in the predictors (in this case the FDR data) as possible. They also provide extra weight for variables that show a high correlation with the response variable, the reference nutrient concentration (Miller and Miller 2005). The most robust regression models will have as few latent variables as possible. The use of mass (kg ha^{-1}) in the regression seems to have introduced variation which could not be explained by the spectral data (lower R^2s values). The slope of the regression lines decreased (Table 8.3 and Figure

8.3), and since slope represents the degree to which the predicted values change relative to the measured values, it should be as close as possible to 1. If the slopes deviates too much from 1 the model may be very sample sensitive (Williams 2001).

Kawamura *et al.* (2009a) have estimated New Zealand hill pasture nutrients (in kg ha^{-1}) with R^2 = 0.78 and RMSEP = 18.75 for N, R^2 = 0.94 and RMSEP = 2.57 for P, and R^2 = 0.81 and RMSEP = 28.41 for K using PLSR and FDR. The results for N and P were improved when analysing CRDR data (R^2 = 0.89 and RMSEP = 17.14 for N, R^2 = 0.94 and RMSEP = 2.43 for P). In Kawamura *et al.* (2009a) the samples analysed had a wider range for both nutrient content and biomass (N: 13.3-192.1 kg ha^{-1}, P: 1.6-36.9 kg ha^{-1}, K: 1.3-294.2 kg ha^{-1}) and were collected from a hill country site of easy slope on one date; while in this study samples were collected from a steeply sloping, hill country site with a sward structure that varied over four seasons of the year.

The present study adds to the body of literature supporting the observation that the accuracy that might be achievable in remotely predicting pasture nutrient content is strongly influenced by the characteristics of the dataset used to build the calibration function. Regression models calibrated against pasture samples containing a wide range of nutrient contents are likely to improve the apparent accuracy of the model, compared to calibrations developed over a narrow nutrient range. Calibrations based on data collected throughout the year (four seasons) offer the possibility of developing one predictive model to cover the seasonal changes in botanical composition and sward structure, which may provide a better representation of the variability found in the field. With more variability in the reference data the predictive model may have less calibration accuracy. Calibrations developed for more complex areas, such as steep hill sites, will also have to deal with more variability, and again might result in inferior calibration accuracy when compared to calibrations developed for less variable swards in lowland and on easy-hill country.

8.3.5. Important wavelengths

The wavelengths detected as significant by the Martens" Uncertainty Test are highlighted in the regression coefficient plots (Figure 8.4). Some wavelengths which presented high values of regression coefficients were detected as non-significant in the

Martens‟ Uncertainty Test; these wavelengths were mainly localized in the extreme parts of the spectrum and have higher noise (low signal-to-noise ratio).

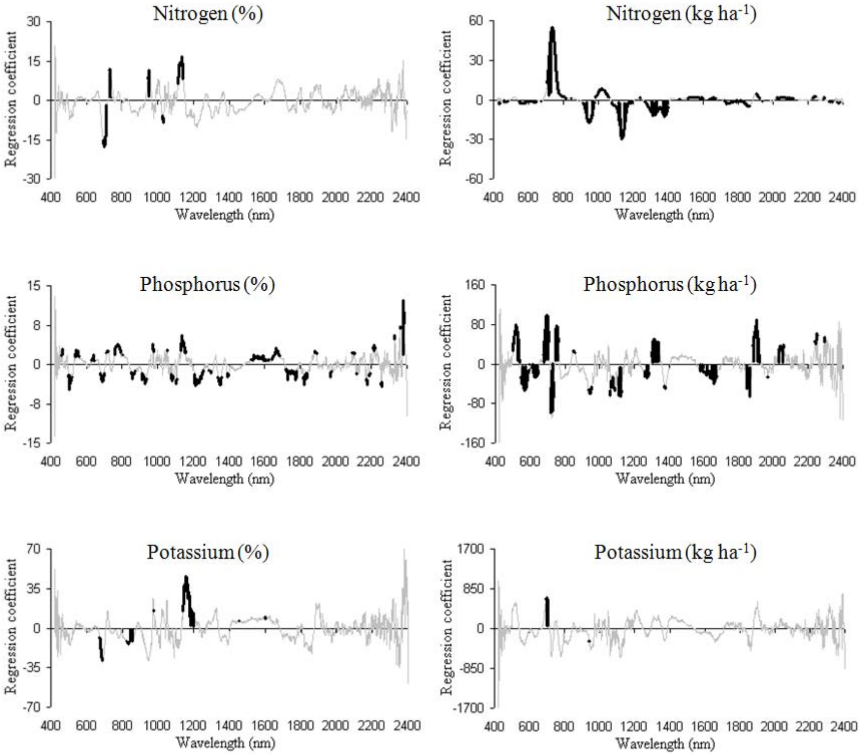

Figure 8.4. Regression coefficients for the PLSR calibration models using cross-validation of the FDR against pasture nitrogen, phosphorus and potassium concentration (%) and mass (kg ha^{-1}). Plotted in black are the significant wavelengths for the regression according to the Martens‟ Uncertainty Test.

Many wavelengths, all over the spectrum, were significantly important in the calibration for P concentration and P and N mass; whereas for N and K concentration and K mass only few wavelengths in the Vis and NIR were significant (Figure 8.4). Predictions for P were based on a greater number of sample observations compared to N and K, meaning more sub-models were created for the cross-validation of the N and K PLSR models. This enabled a more thorough evaluation of the importance of each wavelength. Although several wavelengths were significant in the Uncertainty Test for predicting N mass, there were insufficient sub-models to evaluate the reliability of the wavelengths robustly, as few samples were analysed and only one latent variable was used in the PLSR.

Predictions for all three nutrients had two common significant spectral regions (670-750 nm and 1100-1300 nm). The former interval contains the red-edge, the spectral region which has been related to plant chlorophyll content in numerous studies (Kumar *et al.* 2001). Chlorophyll absorbs light in the Vis wavelengths for photosynthesis, and since N is a key component of chlorophyll, the concentrations of N and chlorophyll are correlated (Donahue *et al.* 1983). P and K nutrients are also related to the photosynthetic process and tissue composition of plants, so spectral reflectance in the Vis is also expected to be correlated to these nutrients (Mutanga *et al.* 2004). The range between 1100-1300 nm is one of the four principal zones in the NIR containing biochemical absorption features. This is dominated by C-H stretch 2nd overtones, features common to all biochemical constituents (Peterson and Hubbard 1992).

The use of the full spectrum for developing calibrations enables a greater number of absorption features to be explored with the potential to increase the quality of the regression models. The negative side is that whereas more information is available, more non-information (in the form of noise) is also present too. PLSR deals with this problem by reducing the predictor variables into fewer latent variables; where the spectral variation relevant to the variation in the reference nutrient dataset is described in the first latent variables, retained in the model, and the data noise is incorporated into the later, discarded (Martens and Naes 1987) variables. Nevertheless, to refine PLSR analysis some studies have proposed wavelength selection. Kawamura *et al.* (2008) investigating the performance of the PLSR with waveband selection found that elimination of redundant (or useless) wavebands suggested that only 2-17% of full-spectrum wavebands in the 400–2350 nm range were important for estimating forage biomass and quality. The Kawamura *et al.* (2008) study was based on 86 samples collected on one date. In more recent work Kawamura *et al* (2009a) reported that 62% of the spectrum (400-2400 nm) analysed with PLSR did not contribute to the prediction of pasture nutrient contents. Because the data were collected on a single date, Kawamura *et al* (2009a) have recommended that further research to include other seasons and a greater diversity of pasture types is required to test the approach further.

In the present study, observing the wavelengths selected as significant by the Martens‟ Uncertainty Test for pasture N, P and K predictions (Figure 8.4), it also was possible to identify bands not contributing to the regression models developed. Variables that are non-significant in the Martens‟ Uncertainty Test display non-structured variation (i.e. noise), and they can be considered useless, or unreliable, variables. Once those variables are removed the resulting model should be more stable and robust (i.e. less sensitive to noise) and the prediction error should decrease (Martens and Martens 2000). Limiting the model to the wavelengths considered significant by the Uncertainty Test, the results (Table 8.3) are generally similar to those presented previously for the whole spectrum between 420-2400 nm (Table 8.3). Overall P predictions were slightly better, and N and K predictions a little poorer. The bias (which is the average value of the residuals) did improve (decreased in value) in all regressions, when using only the wavelengths selected as significant. The improved P predictions may reflect the larger number of samples used in the P regressions, enabling more cross-validation sub-models to be used to identify the importance of each wavelength for the predictions.

Slightly better predictions of pasture P concentration were obtained in our study using 29% of the original 1981 wavelengths analysed. Kawamura *et al.* (2009a) also obtained a better result reducing the number of wavelengths used in the prediction models. They found the important wavebands to predict N, P, K and S were located at 980-1080, 1115-1360, and 1700-1920 nm. The wavelength range 1100-1300 nm was selected in both studies, but whereas the red edge (690-740 nm) was important for N, P and K predictions in our study and for crude protein prediction (and other pasture quality parameters) in the study of Kawamura *et al.* (2008), this wavelength range did not contribute significantly to the prediction of N, P and K in the study of Kawamura *et al.* (2009a). Important wavelengths can also vary depending on the reference nutrient unit (concentration, or mass), for example, in this study, wavelengths at 1100-1200 nm were selected as significant in the Martens‟ Uncertainty Test when analysing K concentration, but not for K mass (Figure 8.4). Moreover, the analyses of different spectral data can generate a different selection of wavebands, as reported in Kawamura *et al.* (2008) when comparing reflectance factors with FDR data. For the prediction of CP in their study, wavelengths in the blue spectral region (430 nm and 450 nm) were selected as important only when analysing reflectance factor data, and wavelengths around 1515-1520 nm were selected only when analysing FDR data. Waveband selection might improve PLSR predictions, but this is not a trivial or easy task. Before any conclusions about the best wavebands for the estimation of particular plant variables can be defined, further research is required.

8.4. Conclusions

This study has examined the use of proximally-sensed hyperspectral reflectance factors between 420 and 2400 nm to predict hill pasture N, P and K concentration and mass. PLSR models using the first derivative of the reflectance (FDR) spectra gave more accurate predictions of pasture P concentrations, followed by pasture K and N concentration. The poor prediction of N concentration may be explained in part by the limited number of observations included in the sample set. The PLSR using the FDR of the full spectrum could explain 47%, 71% and 68% of the variation of pasture N, P and K concentration (%), respectively. The product of concentration and biomass, mass (kg ha^{-1}), added more variation to the reference data but this could not be explained by the PLSR regression models, decreasing the quality of the predictions.

Our study adds to the body of literature that suggests waveband selection might improve PLSR predictions. Further research analysing datasets with a greater number of samples, and samples with a wider range of nutrient contents collected from a greater variety of field sites to ensure a good representation of the range of swards found in the field (such as pasture samples from different seasons) is still required.

There is indeed potential to use proximal sensing techniques to predict nutrients of hill pasture *in situ*. But this potential will only be realised if a substantial dataset representing all the variability found in the field is gathered. This substantial dataset is required to first develop a universal calibration (including all data) and then evaluate, for each situation, if specific calibrations (for specific areas, seasons, or using specific wavebands, etc.) would be more appropriate (increase the predictions accuracy).

CHAPTER 9:

Summary, general discussion, final considerations and future work recommendations

In this Chapter, the major findings of the present research are summarised, discussed with some final reflections, including suggestions for future research.

9.1. Summary, general discussion and final considerations

9.1.1. Chapters 2, 3 and 4

The present research investigated the potential of pasture reflectance spectra, acquired *in situ*, to describe and quantify sward characteristics important in making critical decisions on the management of pastures in New Zealand. Reflectance measurements in the field, were recorded by representing reflectance (the ratio of the reflected flux on a sample surface to the incident flux from the same surface) as a reflectance factor (the ratio of the radiant flux reflected by a sample surface to that reflected into the same reflected-beam geometry by an ideal, perfectly diffuse, standard surface irradiated under the same conditions). New Zealand has changeable weather with rarely still atmosphere and high cloud frequency. These are characteristics which limit the collection of reflectance factors under natural conditions (e.g. sunlight). If the illumination conditions vary between the acquisition of the reference and the target spectra, the resultant reflectance factor spectra will present errors. The Canopy Pasture Probe (CAPP) was developed to allow the acquisition of the pasture‟‟ swards reflectance factor, in the field, independent of the ambient conditions (e.g. cloud cover, wind) (**Chapter 2**). Pasture reflectance factors were acquired in this research using an ASD FieldSpec® Pro FR spectroradiometer, which was attached to the CAPP.

The ASD FieldSpec® Pro FR is a spectroradiometer which covers the spectral range between 350-2500 nm, with spectral resolution of 3 nm for the region 350-1000 nm and 10 nm for the region 1000-2500 nm. It is important to note that spectral resolution (measure of the narrowest spectral feature that can be resolved by the instrument) is different and independent from the spectral sampling interval (spacing between sample points in the spectrum). The sampling interval for the ASD FieldSpec$^{®}$ Pro FR is 1.4 nm for the region 350-1000 nm and 2 nm for the region 1000-2500 nm, with measurements automatically interpolated and reported in 1 nm intervals (one value for 350 nm, one value for 351 nm, etc.).

The CAPP consists of an inverted black bin; a lamp to provide a smooth energy output; and a frame to block the interference from exterior illumination and wind while the pasture spectra are recorded. To acquire reflectance factors, a reference panel needs to be used as a reflectance standard. Spectralon$^{®}$ (Labsphere, Inc.) is worldwide the most common reflectance standard used, but it is an expensive material if a large panel is required. After testing a variety of materials, a matt white ceramic tile, which has a reflectance of 80%, was selected as an alternate reflectance standard for field measurements (**Chapter 3**).

The CAPP increases the utility of proximal sensing techniques under field conditions, especially in places of changeable illumination conditions like New Zealand. There are other challenges for the use of spectroscopy *in situ*, including the surface wetness of pasture canopy (**Chapter 4**). Water can mask some important spectral absorption features and consequently compromise the prediction of pasture parameters from spectra. The ideal would be to collect spectral data without any surface moisture in the pasture, but under field conditions this is rarely the case. Some straightforward approaches to deal with pasture surface moisture were adopted in this research: to avoid collecting spectral data when pasture was likely to be wet (e.g. immediately after rain or irrigation, early in the morning with dew still on the ground) and to use mathematically transformed spectra, including derivatives to reduce the effect of water from the sensed spectra.

9.1.2 Chapters 5, 6, 7 and 8

Under laboratory conditions sensing technologies have been proven very useful for estimating plant biochemistry attributes from dried and ground samples. The challenge is to extend this technology for *in situ* conditions (i.e. standing mixed plant communities under field conditions). To evaluate the ability of proximal sensing for predicting pasture nutrients and botanical composition, reflectance factor data were acquired from a number of lowland dairy and hill sheep pastures in New Zealand, in each of the four seasons of the year (August 2006 to September 2007) using an ASD FieldSpec® Pro FR attached to the CAPP (top-grip).

The relationships between pasture spectra and pasture botanical composition and nutrients content were explored using partial least squares regression (PLSR). PLSR was chosen because of the large amount of data and predictor variables are highly correlated (the information in single wavelengths is usually highly correlated with information in other wavelengths). That means there is redundant information, but rather than select a few of the predictor variables, it is better to reduce their number to a few components (latent variables). Depending on the number of samples available, the accuracy and precision of the PLSR calibration models were evaluated using separate calibration and validation datasets or using the leave-one-out method of full cross-validation.

A review of the research literature indicated that there is not a consensus on the criteria for evaluating the efficiency of calibration of spectral data against measured plant properties (e.g. nutrient content, biomass). The lack of consensus and hence standardization in approaches creates difficulties in comparing the findings of published studies. Frequently, regression results are assessed in terms of coefficient of determination (R^2) and root mean square error (RMSE). Too often R^2, which is the percentage of the variance explained by the regression function, and highly dependent on the range of the samples used in the regression, is used alone. The RMSE, which is a direct estimate of the modelling error expressed in original measurements units, when analysed together with the R^2 provides a more complete view of the error of prediction. However the RMSE does not allow comparison between different parameters. Other statistics used to assess calibration regressions are the regression slope (rate of change in the dependent variable associated with a change in the independent variable), bias (the systematic difference between predicted and measured values) and ratios between standard deviation (SD) of the measured data in the validation dataset and standard error of prediction (SEP) or RMSEP (which enables the comparison between different parameters). In this research, the accuracy and precision of the PLSR models to predict pasture parameters were assessed using R^2, RMSE of prediction or cross-validation (RMSEP or RMSECV), regression slope, bias and the ratio of SD of the measured nutrient concentration or mass to the RMSEP or RMSECV (SD/RMSEP or SD/RMSECV, to provide a complete evaluation of regressions and allow comparison with other published studies. Accurate and precise predictions were those with high R^2, low RMSEP (or RMSECV), slope close to one, bias close to zero, and high SD/RMSEP (SD/RMSECV). To assess which wavelengths were significantly important for the pasture nutrient predictions models a Martens‟ Uncertainty Test, available in The Unscrambler® software, was applied.

(Chapter 5) Data collected to describe pasture botanical composition (expressed as grass, legume and weed proportions), were used to characterise the nature of the pastures at each of the field sites. As a consequence botanical separation of the grass, legume and weed components of the pasture samples did not extend to separating green plant material (e.g. leaves) from non-green plant material (e.g. dead matter, seed heads, etc.). Green plant materials are spectrally different from non-green plant materials. This divergence appeared in the spectral data analysed but could not be taken into account in the botanical composition data. Nevertheless some useful information could be extracted from this study. While some PLSR calibration models could predict pasture grass and legume proportions with reasonable accuracy, the weed component was poorly predicted in all situations. The poor fit of the calibration models might be explained by the narrow range in proportion of weeds in the pasture samples analysed and by the fact that several distinct species (buttercup, catsear, chickweed, etc.) with contrasting leaf morphologies were grouped into a single weed category.

The improved accuracy of the PLSR calibration models for predicting legume and grass proportions from samples collected from dairy lowland pasture plots (e.g. Colyton dataset) appears to be due to the fact that there were few grass and legume species in the dairy pasture, compared with the hill pasture (e.g. Alfredton dataset) where a great diversity of distinct grass and legume species were found. The high botanical diversity found in the hill country site that appeared in the spectral data, was not adequately described by the simple botanical separation.

Of the four seasons, autumn presented the most accurate calibration model (also the best range of grass and legume percentage), winter, spring and summer (summer data available for one site only) calibration models were less accurate, with winter calibrations highly affected by the narrow range of sample data. Quantification of the non-green pasture components, along with separation to pasture species level, might have improved spring and summer predictions of botanical composition.

(Chapter 6) Different mathematical transformations were applied to the reflectance factor spectra before regressing against pasture N, P and K concentrations. In general more accurate predictions were achieved using the first derivative data. Clustering the data according to the season of the year increased the accuracy of predicting the concentrations of the three pasture macro-nutrients. Predictions with reasonable accuracy (R^2 > 0.74 and SD/RMSEP ≥ 2.0) were obtained for N during winter, autumn and summer seasons; P during autumn; and K during summer. The narrow range in %P and %K in pastures in winter appears to be the reason for the low accuracy in the prediction of these nutrients, rather than a weakness in the methodology. Calibrations models derived from spring data explained little variance in the measured nutrient concentration and mass. The reason for that is not fully understood. Some wavelengths which presented high values of regression coefficients were detected as non-significant (useless or unreliable) in the Martens‟ Uncertainty Test. These wavelengths were mainly localized in the extreme parts of the spectrum and have higher noise (low signal-to-noise ratio). Seasonal variations in the important wavelengths selected by the PLSR calibration models to predict pasture nutrient concentrations were observed.

(Chapter 7) First derivative reflectance (FDR) spectra obtained from lowland dairy pasture grown under differential P and N fertiliser applications, were regressed against pasture N, P (also K, Ca, Mg, Na and S) concentration (%) and mass (kg ha^{-1}). Field spectroscopy has the potential to predict pasture nutrient contents, but the quality of the calibrations and hence the prediction is dependent on pasture type, management practices such as fertiliser application, through to the unit of expression of nutrient status. The predictive power of the PLSR models developed in this Chapter varied with each element. Some nutrient were predicted more successfully (e.g. N, Ca and Mg) than others (e.g. P and S). For example higher R^2s and SD/RMSECV resulted for N prediction when the regressions were carried out using nutrient mass (R^2s = 0.61-0.78 and SD/RMSECV = 1.6-2.1) rather than nutrient concentration (R^2s = 0.39-0.63 and SD/RMSECV = 1.3-1.6). Pasture P concentration and P mass predictions were inaccurate (R^2s = 0.31-0.42 and

SD/RMSECV = 1.2-1.3). The importance of separating datasets on the basis of pasture composition when building, evaluating and using prediction calibrations was highlighted when comparing prediction accuracy between pasture fertilised with *added P* (pasture with average legume content = 20%) or *added P+N* (pasture with average legume content = 2%).

(Chapter 8) FDR spectra obtained from hill sheep pasture were regressed against pasture N, P and K concentration (%) and mass (kg ha^{-1}). The PLSR calibration models for % N prediction had the following statistics: $R^2 = 0.47$ and SD/RMSECV = 1.4; for %P: $R^2 = 0.71$ and SD/RMSECV = 1.9; and for %K: $R^2 = 0.68$ and SD/RMSECV = 1.8. The less accurate prediction for N may be explained in part by the limited number of observations included in the sample set. When the wavelengths were limited to those selected by the Martens‟ Uncertainty Test, the accuracy of prediction for pasture % P increased slightly ($R^2 = 0.78$ and SD/RMSECV = 2.0). The PLSR calibration using the FDR of the full spectrum could explain 47%, 71% and 68% of the variation of pasture N, P and K concentration (%), respectively. The product of concentration and biomass, the nutrient mass (kg ha^{-1}), added more variation to the reference data but this could not be explained by the PLSR regression models, decreasing the quality of the predictions. The study presented in this Chapter adds to the body of literature that suggests removal of redundant wavebands might improve PLSR predictions.

A comparison of the best PLSR calibrations for predicting pasture N, P and K concentrations and masses (Chapters 6, 7 and 8) is summarised in Table 9.1.

Table 9.1. Comparison of the best predictions for pasture N, P and K concentration and mass (see Chapters 6 to 8). Predictions with reasonable accuracy (R^2 > 0.74 and SD/RMSEP ≥ 2.0) are highlighted (in bold).

			SD/		**N. of**	**Sample Range**		**Seasons**				**Thesis**
Nutrient	**Unit**	R^2	**RMSECV**	**Sites**	**samples**	**%**	**kg ha^{-1}**	**W**	**Sp**	**Su**	**A**	**Chapter**
N	**%**	**0.83**	**2.4**	**Alf., Col.**	**52**	**2.75-4.85**		**x**				**6**
N	**%**	**0.77**	**2.1**	**Alf., Col., Ati.**	**61**	**1.42-4.42**					**x**	**6**
N	**kg ha^{-1}**	**0.78**	**2.1**	**Col. (*added P*)**	**80**		**54-130**	**x**	**x**		**x**	**7**
N	**%**	**0.76**	**2.0**	**Alf., Bal., Rua.**	**82**	**1.16-4.99**				**x**		**6**
N	%	0.63	1.6	Col. (*added P*)	80	2.99-4.53		x	x		x	7
N	%	0.47	1.4	Alf.	48	1.16-3.80		x	x	x	x	8
N	kg ha^{-1}	0.14	1.1	Alf.	48		4-75	x	x	x	x	8
P	**%**	**0.77**	**2.1**	**Alf., Col., Ati.**	**84**	**0.15-0.52**					**x**	**6**
P	%	0.71	1.9	Alf.	140	0.15-0.49		x	x	x	x	8
P	%	0.48	1.4	Col.	160	0.31-0.79		x	x		x	7
P	kg ha^{-1}	0.41	1.3	Col. (*added P*)	80		6-16	x	x		x	7
P	kg ha^{-1}	0.42	1.3	Alf.	140		13-10	x	x	x	x	8
K	**%**	**0.76**	**2.0**	**Alf., Bal., Rua.**	**93**	**1.00-4.15**				**x**		**6**
K	%	0.68	1.8	Col. (*add.P+N*)	80	1.30-3.93		x	x		x	7
K	%	0.68	1.8	Alf	92	0.90-3.80		x	x	x	x	8
K	kg ha^{-1}	0.49	1.4	Col. (*add.P+N*)	80		39-129	x	x		x	7
K	kg ha^{-1}	0.48	1.4	Alf.	92		0.76-76	x	x	x	x	8

The present research has demonstrated that reasonably accurate pasture nutrient predictions can be attained from fresh, *in situ*, canopy reflectance measurements. The importance of obtaining representative datasets that embrace all the biophysical factors (e.g. pasture type, canopy structure) likely to affect the relationship when building prediction calibrations was emphasized in this research by the different results obtained for the predictions of the same nutrient using different datasets and by the low number of common wavelengths when comparing predictions (c.f. predictions of pasture N, P and K presented in Chapters 6 to 8). The confidence to use a single model to predict multiple nutrients or a model developed for individual nutrients will only come through building a good understanding of the factors likely to influence any one calibration function. Considerable potential exists to use proximal sensing techniques to predict and manage the mineral nutrients of pasture *in situ*. But this potential will only be realised if research can be conducted on substantial field datasets that encompass the major causes of variability in pasture nutrient content and spectral reflectance.

9.2 Future work recommendations

For calibration of spectral reflectance to pasture botanical composition it is recommended that future studies separate living and senescing grass, legume and weed in addition to dissection of grass, legume and weed by species. Detailed work at this development phase may lead to greater utility of the spectral data. For example, whereas the interest in sensing the grass component of a pasture might be to assess persistence of a recently sown cultivar and the legume content to predict feed supply quality and animal growth rates, early detection of invasion of weed species may allow targeted herbicide applications and reduced herbicide use.

For this purpose it would be interesting to test discriminant analysis of the spectral data, a technique to classify new observations into pre-defined classes. In the discriminant analysis, a calibrated dataset is used to create discriminant functions, which are used to predict the class of new samples. The classes could be pastures with different proportions of legume, for example, class 1 = pastures with legume percentage ≥ 20% and class 2 = pasture with legume percentage < 20%.

It has been reported in the literature that the continuum removal technique can improve the relationship between hyperspectral data and plant nutrients, consequently increasing plant nutrient predictions based on spectral data. This improvement was not observed in this present research (Chapter 6). In this study, the continuum removal was applied to the absorption features between 420-518 nm, 550-750 nm, 910-1081 nm, 1116-1284 nm, 1720-1786 nm, 2010-2196 nm and 2222-2378 nm. It would be interesting to test the continuum removal technique applied to other spectral ranges in datasets representing all the variability found in the field.

In general, the calibration models derived from spring data to predict pasture N, P and K in this research were poor. The reason for this is not clear, and further research is necessary to clarify the situation. In spring, pastures are growing very quickly, so a rapid change in biomass is observed. In this study (Chapter 6), there is some indication that prediction of pasture nutrient content might be affected by pasture biomass. It would be interesting to include the pasture biomass as a variable in the PLSR and test if predictions could be improved for the periods when pasture biomass is high (e.g. spring).

Some of the calibrations and predictions presented in this research were based on smaller than the ideal (100-150 samples) datasets. All too often this is also the case found in the literature, although as in the present study, many of these investigations were exploring the potential of the methodology beyond current applications. But, to truly evaluate calibrations and predictions with confidence, and build a calibration to be put into practice, a large dataset with a significant range of spectral and measured data is necessary. Cross-validation is indeed a useful method to test a calibration when there is a shortage of data, but larger datasets would allow independent calibration and validation. A calibration validation based on a properly collected validation dataset is better than cross-validation, because in cross-validation the samples are not truly independent.

Remembering that a good spectral dataset needs to have samples of both pasture quality and quantity that represent all the variability found in the field, then the purposes of a study need to be clear before gathering the data. In the case of collecting data to explore field spectroscopy for estimation of pasture nutrients, it is recommended that the researcher: is aware of all farm practices that affect sward characteristics (e.g. fertiliser applications, grazing, weed control, etc.); is aware of the differences in pasture swards caused by seasons of the year (e.g. different pasture botanical composition); and, only after all this information is known, collects data (> 100-150 samples) that represent all this variation.

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