Sonja Biewer

Hyperspectral field measurements to determine dry matter yield and nutritive value of legume-grass swards

This work has been accepted by the Faculty of Organic Agricultural Sciences of the University of Kassel as a thesis for acquiring the academic degree of Doktor der Agrarwissenschaften (Dr. agr.).

1. Supervisor: Prof. Dr. Michael Wachendorf
2. Supervisor: Prof. Dr. Bernard Ludwig

Defence day: 3. February 2009

Eidesstattliche Erklärung

Hiermit versichere ich, dass ich die vorliegende Dissertation selbständig und ohne unerlaubte Hilfe angefertigt und andere als die in der Dissertation angegebenen Hilfsmittel nicht benutzt habe. Alle Stellen, die wörtlich oder sinngemäß aus veröffentlichten oder unveröffentlichten Schriften entnommen sind, habe ich als solche kenntlich gemacht. Kein Teil dieser Arbeit ist in einem anderen Promotions- oder Habilitationsverfahren verwendet worden.

Preface

This thesis is submitted to the Faculty of Organic Agricultural Sciences of the University of Kassel to fulfil the requirements for the degree Dokter der Agrarwissenschaften (Dr. agr.).

This dissertation is based on three papers as first author, which are published or submitted to international refereed journals. They are included in chapter 4, 5 and 6.

Chapter 1 gives the introduction to all parts of the thesis. Chapter 2 contains the objectives of the work and chapter 3 gives an overview of the basic principles of field spectroscopy.

Chapter 7 considers the results of the chapters 4, 5 and 6 in a general discussion. A general conclusion and the summary is given in chapter 8 and chapter 9.

The following papers contribute to this thesis:

Chapter 4:

Biewer, S., S. Erasmi, T. Fricke and M. Wachendorf. 2008. Prediction of yield and the contribution of legumes in legume-grass mixtures using field spectrometry. Precision Agriculture, Online first. DOI 10.1007/s11119-008-9078-9.

Chapter 5:

Biewer, S., T. Fricke and M. Wachendorf. 2008. Determination of dry matter yield from legume-grass swards by field spectroscopy. Crop Science, submitted.

Chapter 6:

Biewer, S., T. Fricke and M. Wachendorf. 2008. Determination of forage quality in legume-grass mixtures using field spectroscopy. Crop Science, submitted.

Table of contents

Tables

Chapter 4:

Chapter 5:

Chapter 6:

Figures

Chapter 6:

Abbrevations

ADF: acid detergent fiber

CP: crude protein

DM: dry matter

EVI: enhanced vegetation index

ME: metabolizable energy

MPLS: modified partial least squares

NDF: neutral detergent fibre

NDVI: normalized difference vegetation index

NIR: Near infrared reflectance

NIRS: near infrared spectroscopy

PLS: partial least square

REP: red edge position

RPD: residual predictive value

PSE: Partial standard error R^2: Coefficient of determination

SE: Standard error

SEC: standard error of calibration

SECV: standard error of cross validation

SMLR: stepwise multiple linear regression

SR: simple ratio

VI: vegetation index

1-VR: coefficient of determination of cross validation

1 General introduction

Productivity and botanical composition of legume-grass swards are important factors for successful arable farming in both organic and conventional farming systems. A main advantage of legumes is their ability to fix atmospheric nitrogen by legume-rhizobium symbiosis (Boller and Nösberger, 1987). Many detailed studies investigating legume-grass mixtures have shown that high amounts of nitrogen (>300 kg N ha^{-1}) can be fixed by legumes (Loges, 1998; Schmidtke, 1997; Weißbach, 1995). Hence forage legumes in mixture with grass are virtually self-sufficient for nitrogen and can concurrently transfer appreciable nitrogen to the companion grass (Heichel and Henjum, 1991). Another benefit of legumes is their excellent feeding value for animal production (Frame, 1992). In comparison to grass, clover is richer in protein and minerals and the digested nutrients are metabolized more efficiently. Particularly white clover contains lower fibre, is more acceptable to stock and maintains a better digestibility over the season (Frame, 1992; Wilhelmy, 1993). Therefore legume-grass mixtures usually result in increased yield, higher quality and improved seasonal distribution of forage when being compared to pure swards of legumes or grass (Sleugh et al., 2000).

1.1 Site specific determination of canopy parameters of legume-grass mixtures

Growth of legumes can vary strongly through spatial and temporal influences. For example, legume abundance depends on seasonal disturbances, such as cutting, frost and drought damage and can lead to sustained field scale variations in legume content that are only partly explained by the level of seasonal disturbance itself (Schwinning and Parsons, 1996). Hence, continuous mapping of legume distribution in the field could help to understand the processes which affect the abundance of legumes in swards at the field scale and to adapt the management to these processes. Moreover, since yield and proportion of legumes are strongly related to the amount of fixed nitrogen (Høgh-Jensen et al., 2004), frequent information on the status of legume-grass swards could help to predict the nitrogen supply of the soil for arable crops to be grown after the legume-grass mixtures or to direct fertilizer applications. Besides, forage management could be optimized as forage quality is highly affected by the yield and legume composition in the swards.

To analyse spatial distribution of dry matter yield and forage nutritive value and to predict its variability on grassland, systematic manual plant sampling has been used (Bailey et al., 2001; Gottardi, 2008). Although widely used in experiments, these procedures are not applicable to real farm situations because they are too labour intensive. A technical solution for yield monitoring in grassland and forage crops is proposed by a pendulum sensor which works through bending crop-stems and recording, on-the-go, the angle of the suspended pendulum (Ehlert et al., 2003). Another solution is to measure the rate of flow on forage harvesters (Kumhála and Prosek, 2003). However, such measurements are only able to monitor yield, whereas spectral measurements offer more possibilities through a simultaneous detection of several plant parameters, such as biomass (Numata et al., 2007; Schino et al., 2003), plant nitrogen (Lamb et al., 2002; Mutanga et al., 2003), water status (Fitzgerald et al., 2006), vegetation cover (Eastwood et al., 1997) or leaf area index (LAI) (Ray et al., 2006).

A highly accurate application of spectral measurements is the near infrared reflectance spectroscopy (NIRS) which has been successfully used for the detection of agricultural products, foodstuffs, forage and pharmaceutical products (De Boever et al., 1996; Montes et al., 2006; Nie et al., 2008; Norris et al., 1976; Shenk and Westerhaus, 1993). Although the high prediction accuracy of NIRS is comparable with laboratory chemical methods, it still requires the collection and preparation of vegetation samples which is not practicable at field. In contrast remote sensing by satellites or aircraft needs no sample preparation as reflectance data can be automatically collected during the overflight. The challenge of this technique is the coinstantaneous realisation of an adequate spatial and spectral resolution and sufficient frequency of coverage through the return interval. However, field spectral measurements do not suffer such problems, as they can be collected with high spatial and spectral resolution and time and frequency of application can be freely determined. A spectral field sensor with special configuration to detect N deficiency in cereal crops is already available in practice (YARA N-sensor®), but such sensing technique has not yet been tested on grassland (Schellberg et al., 2008).

1.2 Analysis of spectral data

While several studies confirm the use of spectral reflectance data to determine patterns in species assemblages, biomass and nutritive values of grassland communities, techniques to analyse the spectral data vary widely (Cho et al., 2007; Dymond et al., 2006; Locher, 2003; Schmidtlein and Sassin, 2004; Shut et al., 2006).

1.2.1 Vegetation indices

A simple approach is the mathematical transformation by ratio building of vegetation reflectance into dimensionless measures, generally known as vegetation indices (VIs). A common known VI is the simple ratio (SR), which is calculated by the ratio of NIR to red reflectance (Eq. 4.2). The normalized form of SR represents the normalized difference vegetation index (NDVI; Eq. 4.3), which is often used for monitoring natural biomes and agro-ecosystems (Boegh et al., 2002; Box et al., 1989; Ferreira et al., 2003).

Most VIs are calculated by various arithmetic combinations of red reflectance and near infrared reflectance (NIR). While visible red reflectance corresponds to the chlorophyll pigment in leaves which causes considerable photosynthetic absorption of the incoming radiation, NIR is linked to the part of the spectrum where spongy mesophyll and plant cell structural material leads to high reflectance (Jensen, 2000; p. 339). However, there are major limitations with these indices despite their wide application for the analysis of spectral data. Several studies show that VIs can be unstable, varying with soil colour, canopy structure, leaf optical properties and atmospheric conditions (Huete and Jackson, 1988; Middleton, 1991; Qi et al., 1995; Todd et al., 1998). Furthermore, NDVI asymptotically approaches a saturation level after a certain biomass or LAI height (Sellers, 1985; Gao et al., 2000). Other variants of NDVI such as the enhanced vegetation index (EVI) have been developed to correct for soil and atmospheric effects (Eq. 4.4). Besides, EVI is less sensitive to saturation when it exceeds a certain biomass or LAI level (Boegh et al., 2002; Huete et al., 2002). Hence, the improved EVI is more sensitive in detecting seasonal changes among various vegetation formations compared to NDVI (Ferreira et al., 2003; Huete et al., 2006). The red edge position (REP), which marks the inflection point of the ascending part of the reflection curve between red and NIR (Eq. 4.5), is also known to be less sensitive to changes in percentage soil cover and to atmospheric effects (Pu et al., 2003; Huete et al., 2002). The REP has been used as a means to estimate grass/herb biomass (Cho et al., 2007), foliar chlorophyll concentration and content (Lamb et al., 2002; Pinar and Curran, 1996) as well as an indicator of vegetation stress (Horler et al., 1983). Another benefit of the REP over the NDVI is that it is less sensitive to sensor view angle (Blackburn and Pitman, 1999; Clevers et al., 2001). Despite the limitations of VIs, their advantage is the use of a limited range of the whole spectrum which lower the costs for a potential sensor. Hence, for practical purposes the application of VIs to predict vegetation parameters of legume-grass swards is desirable.

During the past decades vegetation indices have been based on either broad wavebands (50-100 nm scale) for example from the satellite-based Landsat Thematic Mapper or short wavebands (10 nm scale) from field-based spectrometers (Hansen and Schjoerring, 2003). Broadband VIs use average spectral information over a wide range resulting in a loss of critical spectral information available in specific narrow (hyperspectral) bands. Therefore further improvement in the prediction accuracy of indices is generally obtained through the use of spectral data from distinct short bands. For example, Starks et al. (2006a, b) demonstrate that narrowband SR perform better in estimating forage neutral detergent fibre (NDF), acid detergent fibre (ADF) and crude protein (CP) of bermudagrass [*Cynodon dactylon (L.)*] pastures when compared to the broadband NDVI and SR. Many recent studies dedicating on the development of hyperspectral VIs have focused on single crops (Hansen and Schjoerring, 2003; Jain et al., 2007; Thenkabail et al., 2004; Zhao et al., 2005). Hence, the development of hyperspectral reflectance ratios for predicting biophysical canopy parameters and nutritive values of legume-grass mixtures remains to be established.

1.2.2 Modified partial least square regression

Besides this relatively simple approach of univariate regression involving VIs, multivariate regression techniques can be used to predict vegetation parameters. The advantage of multivariate regression models is the inclusion of several wavebands into the analysis, resulting in lower losses of spectral information. A technique widely used in laboratory spectroscopy is the analysis of hyperspectral data with modified partial least square regression (Ehsani et al., 1999; Fassio and Cozzolino, 2004; Leardi and Gonzalez, 1998). The MPLS can easily treat data matrices in which each object is described by several hundred variables (Galadi and Kowalski, 1986; Haaland and Thomas, 1988). This method is closely related to principal component regression. But instead of first decomposing the spectra into a set of eigenvectors and scores and regressing them against the response variables as a separate step, MPLS regression actually uses the response variable information during the decomposition process (Geladi and Kowalski, 1986). The partial least square (PLS) regression has been successfully applied to field spectral data for the determination of plant biomass, leaf area index (LAI), nitrogen and chlorophyll concentration and density of wheat canopies (Hansen and Schjoerring, 2003), to assess the effects of different nitrogen applications on a potato crop (Jain et al., 2007) and to calculate within-field variation in crop growth and nitrogen status of rice (Nguyen et al., 2006). Furthermore good results were obtained by PLS predicting biomass of complex grassland sites (Cho et al., 2007) and biomass and its nitrogen content of species rich meadows (Gianelle and Guastella, 2007). Starks et al. (2004) determined forage NDF, ADF and nitrogen concentrations of bermudagrass with MPLS and found that it could explain 63 to 76% of the variability expressed in the reference data. However, the great potential of MPLS, offering the use of the whole hyperspectral range, still remains to be examined for the estimation of biomass and forage quality variables from legume-grass swards.

1.2.3 Stepwise multiple linear regression

Although MPLS seems to be a powerful method for the analysis of large data sets, it is not practical for livestock managers to predict yield and forage quality variables using an expensive, full range spectrometer. An approach to reduce the range could be the analysis of stepwise multiple linear regression (SMLR), as it enables a few wavelengths to be extracted from the full dataset to create a prediction model (Kokaly and Clark, 1999; LaCapra et al., 1996). This methodology is based on laboratory techniques developed in the agriculture industry for rapid estimation of forage quality from the reflectance spectra of dried and ground foliage (Norris et al., 1976). Problems known with this method are the potential of overfitting, multicollinearity and waveband selection that fail to correspond with known absorption bands (Curran et al., 1992; Grossman et al., 1996). Nevertheless, to identify combinations of wavelengths most highly correlated with canopy chemistry and biomass, several studies have used SMLR with satellite (Huang et al., 2004; Serrano et al., 2002) and field data (Nguyen et al., 2007). In addition, recent research has demonstrated that optimal information to quantify characteristics of different plant species is present in a few specific wavebands (Blackburn et al., 1999; Starks et al., 2008; Thenkabail et al., 2004). Hence, SMLR may be used to identify spectral regions that are highly correlated with vegetation parameters of legume-grass swards to build accurate prediction models by only a few wavebands.

2 Research objectives

The objectives of this study were to evaluate if field spectral measurements can be used to predict dry matter (DM) yield and nutritive values of legume-grass swards across a wide range of legume species, legume proportion and growth stage.

In a first attempt two experiments were conducted in a greenhouse under controlled conditions to allow the potential of field spectroscopy to be assessed for estimating the DM yield of legume-grass swards. Spectral measurements were made with artificial sources of illumination to exclude interference from effects such as wind, passing clouds and changing angles of solar irradiation. Therefore, the results obtained were free from the kind of interference one could expect under field conditions. This initial investigation was then evaluated over two years in a field experiment with the similar legume-grass swards. Finally, field spectral measurements of the second year of field experiment were used to predict metabolizable energy (ME), ash, crude protein (CP) and ADF of legumes-grass swards.

The specific objectives of this investigation were:

i) to determine if the vegetation indices, SR, NDVI, EVI and REP are appropriate indicators to detect DM yield of legume-grass swards and the proportion of legume in the sward.

ii) to evaluate if the development of two-waveband reflectance ratios, based on signals at specific narrowbands enable the estimation of ME, ash, CP and ADF.

iii) to develop reflectance algorithms for the prediction of DM yield and the forage quality constituents ME, ash, CP and ADF based on the total reflectance in the visible and near infrared wavelength ranges using modified partial least square regression and stepwise multiple linear regression.

iv) to reduce the hyperspectral data range for the prediction of DM yield, ME, ash, CP and ADF to a few informative bands in order to improve the practical applicability.

3 Basic principles of field spectroscopy

Incident optical radiation can be reflected, transmitted or absorbed by material. Spectroscopy is a widely used method that measures light reflectance to predict quality and quantity parameters of distinct materials. Within field spectroscopy the solar irradiation is used in the range between 400 nm and 2500 nm.

3.1 The Beer-Lambert Law

The fraction of reflected irradiation is the part of energy which is measured by the sensor, but it is strongly dependent on the rate of absorbed radiation. Absorption by a constituent in materials is a linear function of its concentration and the path length of radiance through the material. It is described in the Beer-Lambert law, usually written as:

$$A = a(\lambda) * b * c \tag{3.1}$$

where A is the measured absorbance; $a(\lambda)$ the wavelength-dependent absorptivity coefficient; b the path length; c the concentration of the constituent.

The important feature of this relationship is the possibility of measuring the concentration of the constituent directly from the amount of absorbed irradiance. However, the linearity of the Beer-Lambert Law is limited by chemical and instrumental factors. Chemical limitations comprise deviations in absorptivity coefficients at high concentrations due to electrostatic interactions between molecules in close proximity, scattering of light due to particle structure in the material as well as fluorescence and phosphorescence (spontaneous and delayed release of energy respectively) of the material. Instrumental factors are non-monochromatic radiation and stray light (Miller, 2001). In these cases the relationship between the concentration of the constituent and measured absorption must be calculated by using empirical models. For the development of these models usually the whole concentration range of the constituent is needed (Günzler and Gremlich, 2003).

3.2 Fundamentals of light-plant interaction

On atomic and molecular levels, respectively, several mechanisms are linked to absorption processes.

In the visible light region electronic processes are dominating. These processes involve absorption of photons with specific energy contents or wavelengths, respectively that causes an electron jump from a lower energy state to an electron shell at a higher energy state (Clark, 1999). An example for electronic absorption by vegetation is the photosynthetic process. Light is used by chlorophyll and other pigments for the incorporation of carbon dioxide and water into the energy-rich sugar that can be metabolized by the plants (Jensen, 2000):

$$6CO_2 + 12H_2O \rightarrow C_6H_{12}O_6 + 6H_2O + 6O_2 \uparrow \qquad (3.2)$$

Chlorophyll *a* and *b* are the most important plant pigments absorbing blue and red light: chlorophyll *a* at wavelengths of 430 and 660 nm and chlorophyll *b* at wavelengths of 450 and 650 nm (Curran, 1983; Schilling, 2000). Other pigments such as carotenes, xanthopyll, anthocyanin and phycoerythrin are also present in the plant cells, but usually masked by the abundance of chlorophyll pigments. When a plant undergoes senescence in the fall or encounters stress, the chlorophyll pigment disappears, allowing the carotenes and other pigments to become dominant (Jensen, 2000). Depending on the plant species and the amount and nature of the occurring pigments, the absorption intensity in the visible light differs between 70% and 95% of the solar irradiation (Hildebrandt, 1996). Normally green light is more reflected, resulting in a typical reflection maximum at about 500 nm (Figure 3. 1).

In the near infrared region, reflection processes on plant surfaces dominate, as shown in Figure 3. 1. This prevents proteins from denaturation (Jensen, 2000). Thereby the spongy mesophyll layer in a green leaf controls the amount of near infrared energy that is reflected by the internal scattering at the cell wall-air interfaces within the leaf (Gausmann et al., 1969). The amount of near infrared energy absorbed by plants is responsible for vibrational and rotational processes of valence bonds in asymmetric molecules. At distinct energy levels, the atomic units held by bonds are set into motion, either as a back and forth vibration and/or as a

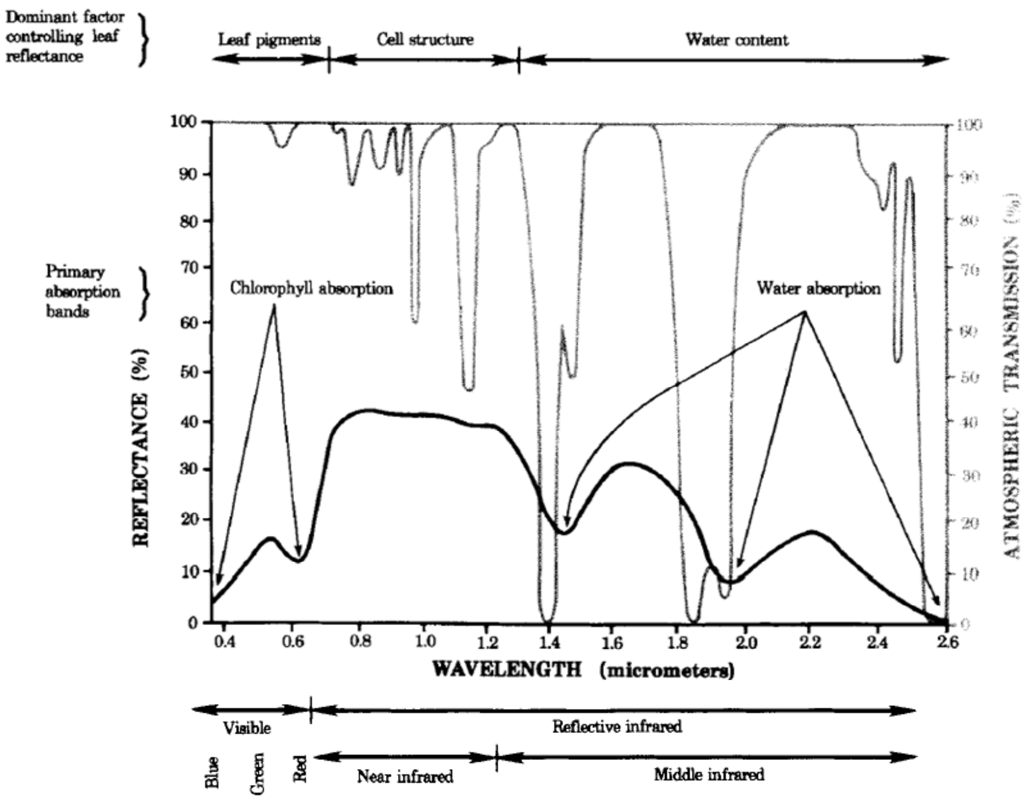

Figure 3. 1: The dominant factors controlling leaf reflectance of healthy, green vegetation for the wavelength interval 400-2600 nm (Source: Jensen, 2000).

rotation. The frequencies that these molecules absorb are usually lower then those offered by visible light and depend on the strengths of the bonds and the masses of atoms or ions participating in the movement. Given a molecule composed of n atoms, there exist 3n minus 6 fundamental vibrations or rotations, respectively. Concurrently, additional vibrations at higher frequencies appear. These comprise combinations of fundamental vibrations as well as overtones, which are fixed multiples - i.e. 1/2, or 1/3 - of the fundamentals. Normally the absorption of these combinations and overtones is weaker.

The most common absorption bands in the near infrared result from the OH, CH, NH and CO functional groups (Rudzik, 1993). Especially water, present in the atmosphere and in the plant canopy, shows strong absorption features. Water in the atmosphere creates the four major absorption bands at 970, 1119, 1450 and 1940 nm (see Figure 3. 1). However, there is also a strong relationship between the irradiance and the amount of water present in the plant canopy. Water in plants absorbs incident energy between the absorption bands of atmospheric water with increasing strength at longer wavelengths. Reflectance peaks occur at about 1600 and 2200 nm (Jensen, 2000). The greater the turgidity of leaves, the lower the reflection of the near infrared light. Conversely, as the moisture content of leaves decreases, reflectance in the near infrared region increases substantially (Jensen, 2000).

Further plant constituents such as protein, carbohydrates, cellulose, lignin and fat, containing different functional groups, show broad absorption bands in the near infrared region. In most instances these absorption bands are masked by water absorption and overlap each other. Thus, a direct assignment of a wavelength to a certain constituent based on the original spectrum is nearly impossible (Socrates, 2001).

4 Prediction of yield and the contribution of legumes in legume-grass mixtures using field spectrometry

Abstract Productivity and botanical composition of legume-grass swards in rotation systems are important factors for successful arable farming in both organic and conventional farming systems. As these attributes vary considerably within a field, a non-destructive method of detection while doing other tasks would facilitate more targeted management of crops and nutrients in the soil-plant-animal system. Two pot experiments were conducted to examine the potential of field spectroscopy to assess total biomass and the proportions of legume, using binary mixtures and pure swards of grass and legumes. The spectral reflectance of swards was measured under artificial light conditions at a sward age ranging from 21 to 70 days. Total biomass was determined by modified partial least squares (MPLS) regression, stepwise multiple linear regression (SMLR) and the vegetation indices (VIs) simple ratio (SR), normalized difference vegetation index (NDVI), enhanced vegetation index (EVI) and red edge position (REP). Modified partial least squares and SMLR gave the largest R^2 values ranging from 0.85 to 0.99. Total biomass prediction by VIs resulted in R^2 values of 0.87 to 0.90 for swards with large leaf to stem ratios; the greatest accuracy was for EVI. For more mature and open swards VI-based detection of biomass was not possible. The contribution of legumes to the sward could be determined at a constant biomass level by the VIs, but this was not possible when the level of biomass varied.

Keywords: Field spectroscopy • Modified partial least squares (MPLS) regression • Stepwise multiple linear regression (SMLR) • Vegetation index.

4.1 Introduction

Biomass production of legume-grass swards is affected by several properties that vary within a field, such as soil moisture concentration, texture, nutrient supply and temperature (Ledgard and Steele, 1992). The site-specific detection of total biomass within the growing period of plants could help in understanding the reasons for poor productivity in some areas and to adapt management to the particular needs of plants. Furthermore, not only poor but also optimal productivity is important to monitor as it provides an insight into the spatial variation of swards and a better understanding of ecological processes and patterns. Legumes with their ability to fix nitrogen contribute considerably to the nutrient supply in forage production (Ledgard and Steele, 1992, Wachendorf et al., 2004), which is of particular importance in organic agriculture, where they contribute 20 to 50% to the arable farm area. Legume-grass swards are usually grown as short-term grassland for 1 to 3 years in a crop rotation system. They are managed by cutting and used for silage or fresh fodder. The yield and proportion of legumes are strongly related to the amount of fixed nitrogen (Høgh-Jensen et al. 2004). Therefore, frequent information on the status of legume-grass swards could help to direct fertilizer applications and to predict the nitrogen supply of the soil for arable crops in the field to be grown after the legume-grass mixtures.

One method of detecting total biomass in the field is to record and map the yield of harvested plants by techniques that measure the rate of flow on forage harvesters (Kumhála et al., 2003). However, such measurements are inevitably destructive and restricted to the time of harvest. Another approach is to predict the amount of green biomass in grassland using spectral measurements, which Künnemeyer et al. (2001) assessed by a simple, portable reflectometer. This method is more flexible as it can be used on various agricultural machines and it also provides non-destructive estimation of other plant parameters, such as plant nitrogen (Lamb et al., 2002; Mutanga et al., 2003; Nguyen et al., 2006) and water status (Fitzgerald et al., 2006), vegetation cover (Eastwood et al., 1997), leaf area index (LAI) (Ray et al., 2006) or biomass (Numata et al., 2007; Schino et al., 2003).

The detection of total biomass of grassland ecosystems by spectral reflectance has been described in several studies (Boschetti et al., 2007; Friedl et al., 1994; Ikeda et al., 1999; Numata et al., 2007; Schino et al., 2003). Most studies used complex grassland types and information from satellite-based sensors that were not synchronized with harvesting of the swards. The majority of studies investigated ecosystems with low biomass production or obtained models with only a poor accuracy of prediction.

The analysis of reflectance data can be done using modified partial least squares regression (MPLS). Although this method can handle several hundred variables to describe an object (Haaland and Thomas, 1988; Nguyen et al., 2006), for practical implementation of a spectral approach at the field scale hyperspectral measurements are too expensive. In general, it is acceptable to use a small range only of the whole spectrum, which is commonly the NIR and the red light. Stepwise multiple linear regression (SMLR) enables a few wavelengths to be extracted from the full dataset to create a prediction model (Curran et al., 1992). The SMLR can be used to identify wavelengths in the spectra where reflectance is correlated with biomass. Those wavelengths can then be applied to estimate biomass in other samples. Also spectral vegetation indices (VIs) use a limited range only of the whole spectrum (Zha et al., 2003); most are based on various arithmetic combinations of red and near infrared reflectance values. Visible red reflectance corresponds to the part of the spectrum where the chlorophyll pigment in leaves causes considerable photosynthetic absorption of the incoming radiation. Near infrared reflectance is linked to the part of the spectrum where spongy mesophyll and plant cell structural material leads to high reflectance (Jensen, 2000; p. 339). A widely used VI is the simple ratio (SR), which is calculated by the ratio of NIR to red reflectance. The normalized form of SR represents the normalized difference vegetation index (NDVI), which is often used for monitoring natural biomes and agro-ecosystems. The limitation of these indices is their sensitivity to variation in the canopy background; this is particularly so when the canopy background brightness is intermediate (Huete, 1988). By contrast, red edge position (REP) and the enhanced vegetation index (EVI) are known to be more insensitive to changes in percentage soil cover and to atmospheric effects (Pu et al., 2003; Huete et al. 2002). The REP marks the inflection point of the ascending part of the reflection curve between red and NIR. A simple but robust method to calculate the REP is a four-point interpolation, which assumes that the reflectance curve at the red edge can be simplified to a straight line centred near the midpoint between the reflectance in the NIR at about 780 nm and the reflectance minimum of the chlorophyll absorption feature at about 670 nm (Dawson and Curran, 1998; Pu et al., 2003). The EVI was developed to optimize the vegetation signal in areas of large amounts of biomass and to improve vegetation monitoring by reducing the canopy background signal and atmospheric effects (Huete et al., 2002).

The primary objective of the present investigation was to examine the relations between reflected light in legume-grass swards and their total biomass and the proportion of legumes based on dry matter (DM). Two experiments were conducted in a greenhouse under controlled conditions. Measurements were made with artificial sources of illumination to exclude interference from effects such as wind, passing clouds and changing angles of solar irradiation. Therefore, the results obtained are free from the kind of interference one could expect under field conditions. The controlled conditions allow the potential of field spectrometry for estimating dry biomass of legume-grass swards to be assessed. This initial investigation was essential to evaluate this technology for future application under field conditions. Eventually, this approach should enable the determination of biomass and the proportion of legumes in the field so that an objective decision can be made on the need to improve the sward by oversowing, together with an estimate of the amount of atmospheric nitrogen fixed by the legumes (Høgh-Jensen et al., 2004). As the nitrogen partly accumulates in the soil and thus is available for the following crops, a more targeted management of these crops is possible by adjusting fertilizer application, sowing rate and choice of crop and cultivar accordingly.

The following questions were addressed in this study:

i) What is the maximum spectral information needed to detect total biomass of legume-grass swards based on the total reflectance in the visible and near infrared wavelength ranges. In addition, which wavelengths are most relevant for biomass prediction?

ii) Are the common vegetation indices, SR, NDVI, EVI and REP, based on signals at specific wavelengths appropriate indicators to determine total biomass of legume-grass swards?

iii) Do reflection characteristics differ among legumes and grass and do they enable the proportion of legume in the sward to be detected?

4.2 Material and Methods

4.2.1 Experimental design

Two pot experiments were carried out in a greenhouse during the year 2004/2005 and 2005/2006 at the University of Kassel (51°20 N, 9°51 E), Germany. In addition to pure swards of red clover (*Trifolium pratense* L.), white clover (*Trifolium repens* L.) and lucerne (*Medicago sativa* L.) they were each combined to form binary mixtures: in the first experiment with perennial ryegrass (*Lolium perenne* L.) and in the second experiment with annual ryegrass (*Lolium multiflorum* Lam. ssp. *alternativum*) (Table 4. 1). To compare swards at different stages of growth, the sowing of seeds was repeated four times at intervals of two weeks (first experiment used only the binary mixtures) and they were all harvested on the same date. The sowing was done manually with a distance between the rows of 12 cm and at a sowing depth of 0.5 cm. The pots were filled with homogenized loamy soil (3.6% sand, 73% silt, 23.4% clay and 2% humus). Soil analysis indicated optimum levels of phosphorus, magnesium and potassium and a pH-value of 6.7. No fertilizers were applied.

4.2.2 First experiment

Eight experimental swards, including four replicates, were sown on 08.11.2004 (Table 4. 1). To determine the effects of growth stage, the sowing of binary mixtures was repeated four times at intervals of two weeks and the pots were harvested on a single day (13.01.2005), which was 21, 35, 49 and 63 days after sowing. The swards were grown in wooden pots of 0.119 m × 0.119 m × 0.2 m. The minimum temperature at night was 10 C° and 18 C° at day. All swards were illuminated for 12 h per day by artificial light.

Table 4. 1: Species, cultivars and seed rates used in experiments 1 and 2.

Treatment	Cultivar	Seed rate legume/grass; kg ha^{-1}				
Experiment 1						
Perennial ryegrass (PG)	Liflora	0/20				
Red clover/PG	Tamara	2/20	8/20	8/0		
White clover/PG	Klondyke	4/20		4/0		
Lucerne/PG	Daisy	16/20		16/0		
Experiment 2						
Annual ryegrass (AG)	Liflora	0/20				
Red clover/AG	Pirat	2/20	4/20	6/20	8/20	8/0
White clover/AG	Klondyke	1/20	2/20	3/20	4/20	4/0
Lucerne/AG	Daisy	4/20	8/20	12/20	16/20	16/0

PG: perennial ryegrass; AG: annual ryegrass.

4.2.3 Second experiment

Sixteen experimental swards were investigated with three replicates and four different growth stages. There were four pure swards and twelve different binary legume-grass mixtures which were sown on 03.11.2005 (Table 4. 1). The sowing of all treatments was repeated four times at intervals of two weeks so that in total 192 swards were examined. An early cut was done for each seeding interval after 9 weeks to reduce the proportion of weeds. The swards were harvested on a single day on 15.03.2006, so that the growth period between the two cuts was for 28, 42, 56 and 70 days. The treatments were grown in wood pots, with dimensions of 0.16 m × 0.16 m × 0.2 m.

4.2.4 Spectral data collection

One day before harvest all swards were measured with a field spectrometer in a dark room using a quartz tungsten halogen lamp (JCV 14.5V – 50W C) to illuminate the swards. The FieldSpec® Pro JR (Analytical Spectral Devices, CO, USA) field spectrometer measured light energy reflected from swards in the range 350 to 2500 nm. Three detectors were used to measure this range. One measured the visible to near infrared portion of the spectrum from 350 nm to 1000 nm with a spectral resolution of 3 nm, and two others measured the short-wave infrared reflectance from 1000 nm to 2500 nm with a spectral resolution of 30 nm. The spectral reflectance was then interpolated by the analytical spectral devices (ASD) software RS_3^{TM} to produce readings at an interval of 1 nm. The field of view

(FOV) was 25° for the sensor optic. To measure reflectance of the incident light on the swards the sensor was stabilized on a tripod at a height of 0.7 m for the first experiment and at 0.8 m for the second one. Spectral calibrations were done after every 6th measurement using a 99% Spectralon panel (Labsphere, Inc., North Sutton, NH, USA). The spectrometer automatically calculated percentage plant reflectance by dividing plant sample reflectance by reflectance of the white standard panel. The dark current, generated by thermal electrons within the spectrometer, was subtracted automatically from the radiometric signal at each calibration session. Each radiometric data point represented a mean of 4 measurements consisting of 10 replicated scans for each. In both experiments the total above-ground biomass was harvested shortly after the spectral measurements were made, and for each sward the yield was separated into fractions of grasses, legumes and weeds. After separation the samples were dried at 65 °C for 48 h.

4.2.5 Analysis of spectral data

Prior to spectral analysis, the spectra were smoothed using eleven convoluting integers and a polynomial of degree five (Savitzky-Golay, 1964; Erasmi and Dobers, 2004). Wavelengths from 350 to 399 nm and from 2401 to 2500 nm, respectively, were excluded from the calculations, as instrument noise in these regions resulted in coefficients of variance greater than 0.1 for the four averaged measurements (Erasmi and Dobers, 2004). Finally, three types of spectral analysis were used to estimate total sward yields:

i) Modified partial least squares (MPLS) regression. This method is used to reduce the large number of measured collinear spectral variables to a few uncorrelated latent variables or factors (Cho et al., 2007). As in multiple regression, the main purpose of MPLS is to build a linear model:

$$y = b_1 x_1 + b_2 x_2 + ... + b_n x_n + e \qquad (4.1)$$

where y refers to the response variable (total biomass in this study), x is the predictor variable (here the spectral wavelengths reduced to independent factors), b indicates the regression coefficients and e are the residuals (Geladi and Kowalski, 1986). Development of the MPLS equation involved smoothing and calculating the first-order derivative of the spectra over a distance of 4 nm. Spectral data from the first and second experiments were divided into six and four groups, respectively, for cross validation. This procedure was used for calibration, which avoids the need for separate validation and calibration sets (Terhoeven-Urselmans et al., 2006). Outliers were removed from each local calibration by two passes through an elimination filter. Outliers were defined as samples with a spectrum outside the population spectra (H-outliers) or as those for which the difference between the reference and the predicted value was much larger than the standard error of cross validation (t-outliers). The limits were set to 10 (H-outliers) and 2.5 (t-outliers) as suggested by Tillmann (2000), resulting in 2 and 9 t-outliers in experiments 1 and 2, respectively, and 8 t-outliers for the combined data.

ii) Stepwise multiple linear regression (SMLR) was performed to select those wavelengths that are most strongly correlated with the reference values and to develop an equation with fewer variables compared to the MPLS. The addition of wavelengths to the equation was continued until the F value fell below 7 (WinISI III Manual, 2005).

iii) Finally, common vegetation indices were calculated from the spectral data, simple ratio (SR), normalized difference vegetation index (NDVI), enhanced vegetation index (EVI) and red edge position (REP), using the following equations:

$$SR = \frac{R_{940}}{R_{640}} \tag{4.2}$$

$$NDVI = \frac{R_{\mathrm{NIR}} - R_{\mathrm{RED}}}{R_{\mathrm{NIR}} + R_{\mathrm{RED}}} \tag{4.3}$$

$$EVI = G\left[\frac{R_{\mathrm{NIR}} - R_{\mathrm{RED}})}{(R_{\mathrm{NIR}} + C_1)(R_{\mathrm{RED}} - C_2)(R_{\mathrm{BLUE}} + L\ )}\right] \tag{4.4}$$

$$REP = 700 + 40\left[\frac{\left(\frac{R_{670} + R_{780}}{2}\right) - R_{700}}{R_{740} - R_{700}}\right] \tag{4.5}$$

where R is reflectance value; NIR the range of reflectance from 800 to 900 nm; Red the range of reflectance from 650 to 700 nm; Blue the range of reflectance from 450 to 500 nm; L the canopy background adjustment that addresses nonlinear, differential NIR and red radiant transfer through a canopy (here L=1); C_1 and C_2 the coefficients of the aerosol resistance term, which uses the blue light to correct for aerosol influences in the red band (here C_1 = 6, C_2 = 7.5); G the gain factor to limit the EVI value to the -1 to +1 range (here G = 2.5; Huete et al., 2002).

Regression analysis was used to estimate the relation between the VIs and total biomass and species proportion using the GLM and NLIN procedures of SAS 9.1 (SAS Institute, 2002-2003). The MPLS and SMLR analyses were performed using WinISI III (Infrasoft International, LLC. FOSS) software package.

4.3 Results

4.3.1 Experiment 1

All swards examined at the time of harvest were at the stage of early stem elongation. Swards grown for 63 days after sowing had an almost closed canopy with, on average, 4% of the area with soil exposed (BBCH stage 29 for grass and BBCH stage 22 for legumes; Meier, 2001). The 49 day old swards were at the beginning of row closure (BBCH 25 for grass and BBCH 19 for legumes) and there was more bare soil (approximately 13%). The 35 and 21 day old swards had a large proportion of visible soil; approximately 50% and 84%, respectively. While the grass of the 35 day old swards had begun to tiller (BBCH 19/20) and the legumes developed their third or fourth leaf (BBCH 13/14), the grass and legumes of the youngest swards were still developing their first leaves (BBCH 12/13 and BBCH 10, respectively).

The proportion of legumes varied from 80% in the pure swards to 6% in the mixtures. By contrast, the proportion of grass was always greater; it gave a yield of 86% in the pure swards reducing to 22% in the mixtures (Table 4. 2).

As swards were investigated at different stages of development, total biomass varied from 2.1 g m^{-2} in the youngest swards to 170.9 g m^{-2} in the oldest swards

Table 4. 2: Range of total biomass (g DM m^{-2}), proportion of legumes, grass and weeds (% of DM) and of proportion of visible soil (% of sward cover) in experiments 1 and 2.

Treatments	N	Total biomass		Legumes		Grass		Weeds		Visible soil[§]	
		Min.	Max	Min.	Max.	Min.	Max.	Min.	Max.	Min.	Max.
Experiment 1											
Grass (G)	4	94.2	134.3	0.0	0.0	72.0	85.8	14.2	28.0	5.8	8.6
Red clover (R)	4	116.6	161.6	50.7	62.3	0.0	0.0	37.7	49.3	1.6	2.2
White clover (W)	4	125.8	163.5	46.9	58.2	0.0	0.0	41.8	53.1	0.5	1.1
Lucerne (L)	4	115.7	142.5	62.1	79.8	0.0	0.0	20.2	37.9	2.1	4.0
RG	32	2.1	170.9	6.1	43.5	42.1	85.2	3.1	50.2	0.9	88.7
WG	16	3.7	122.9	13.7	21.2	21.9	80.2	4.5	28.0	3.0	89.6
LG	16	5.4	132.2	26.2	47.0	39.4	64.8	3.8	27.4	1.9	81.4
Experiment 2											
G	12	27.5	164.2	0.0	0.0	95.9	100.0	0.0	4.1		
R	10	26.3	222.5	66.7	91.5	0.0	0.0	8.5	33.3		
W	12	31.9	186.8	38.8	83.6	0.0	0.0	16.4	61.2		
L	11	25.0	155.4	60.0	90.3	0.0	0.0	9.7	40.0		
RG	48	39.4	175.7	11.6	40.9	61.4	92.2	0.0	5.6		
WG	48	103.6	296.1	7.5	21.1	84.6	96.8	1.6	7.1		
LG	47	96.8	194.4	13.7	45.7	74.8	98.5	0.9	6.3		

[§] Data from Himstedt (personal communication); G: grass; R: red clover; W: white clover; L: Lucerne; RG: red clover-grass; WG: white clover-grass; LG: lucerne-grass; DM: dry matter.

(Table 4. 2). Differences in total biomass were caused mainly by the legumes, as the grass yield did not differ greatly among treatments.

The reflectance values from the spectral measurements show a characteristic pattern for herbaceous plant canopies, with high absorption in the visible light and short-wave infrared radiation and large reflectances in the near infrared. Pure swards have very different NIR reflectances, but the differences are minor for the visible light (Figure 4. 1A). Although total biomass of the 63 day old pure red and white clover swards is almost similar, their NIR reflectances are very different. By comparison to sward canopies, bare soil shows a distinct spectral signature with low reflection in the NIR (Figure 4. 1B). As the youngest swards had very small biomass, their spectral signature resembles closely that of bare soil. With advancing age, signals of bare soil wane and NIR reflectance intensifies.

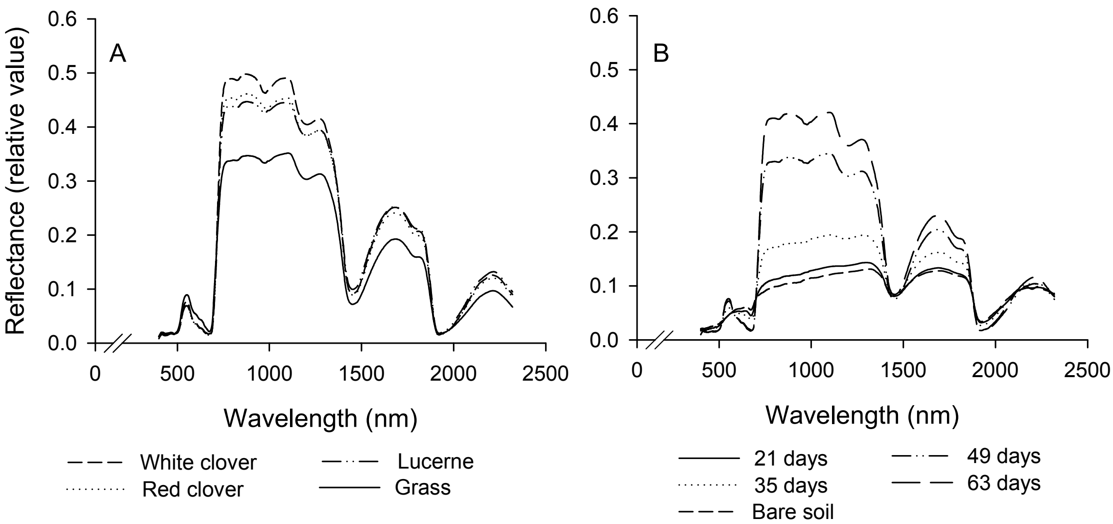

Figure 4. 1: Spectral signatures of: (A) pure swards at an age of 63 days and of (B) white clover-grass mixtures at different sward age (21, 35, 49 and 63 days after sowing) and of bare soil in experiment 1.

The MPLS regression predicts total biomass with a coefficient of determination (R^2) of 0.99 and a standard error of calibration (SEC) of 6.1 g dry biomass m^{-2} (Figure 4. 2, Table 4. 3). In the cross validation, the coefficient of determination (1-VR) is 0.95 and the standard error (SECV) is 12.0. Two outliers were eliminated, which were pure grass samples. Legume-specific calibrations result in improved accuracies of prediction for all mixtures (Table 4. 3). Generally, the regression equations for MPLS estimates are close to the y=x line. The equation derived from SMLR comprises five wavelengths representing blue (403 nm), red (747 nm) and near infrared (938, 993, 929 nm) regions of the measured spectral range (Table 4. 4). The resulting model gives an R^2 of 0.97 and an SEC of 10.1 g DM m^{-2} (Table 4. 3).

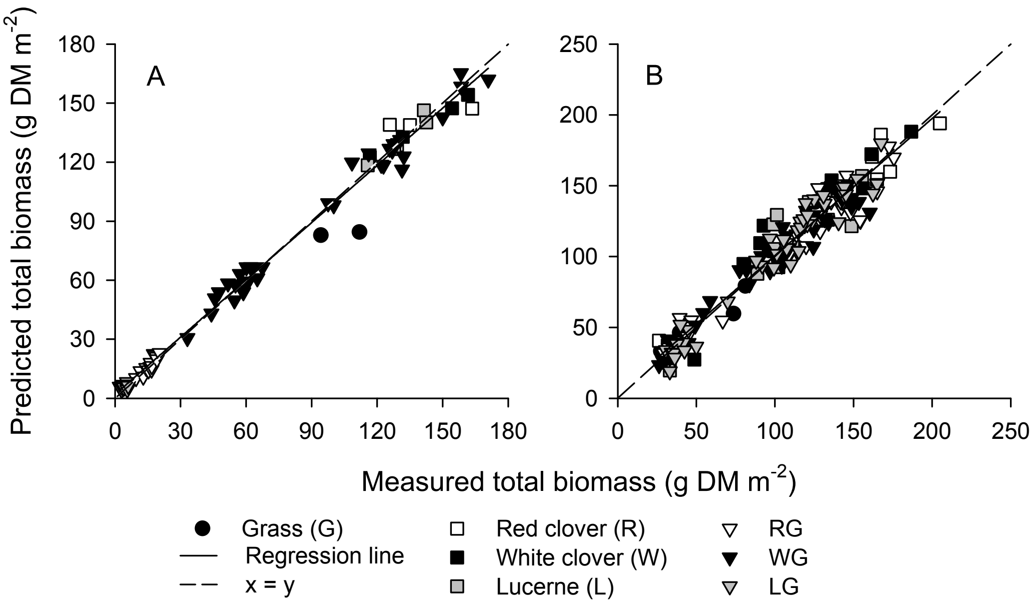

Figure 4. 2: Relationship of the common data set between MPLS-predicted and measured total biomass (g DM m^{-2}) of swards in: (A) experiment 1 and (B) experiment 2 (for regression statistics see Table 4. 3).

The VIs are positively correlated with the total biomass of swards (Figure 4. 3, Table 4. 5). Based on all VIs, the coefficients of determination for the common calibrations of all 80 swards are somewhat less (0.87 to 0.90) than the legume-specific calibrations (0.90 to 0.98). However, an almost vertical slope of the exponential curves in the case of NDVI and REP at high levels of total biomass prevents an accurate estimation of yields. By contrast, the linear or almost linear relation between total biomass and SR and EVI, respectively, facilitates the prediction across the whole range of biomass. Separate calibrations for mixtures with the same legume species improve the accuracy of the model; the standard errors (SE) for REP are particularly small (5.9 to 12.7 g m^{-2}). Compared to the common calibration, calibrations including only mixed swards reduce the SE to 14.2 g m^{-2}, averaged over all VIs. By contrast, calibrations based on the pure swards generally fail, except for lucerne, but here the sample size is small with n=4. The difficulties with pure swards are also evident from the large partial standard errors (PSE) from the application of the common calibration to each plant species separately. Values for pure swards are mainly larger than for mixtures, which is particularly so for pure grass with 58.7 (EVI) to 92.8 g m^{-2} (REP). It is remarkable that for all VIs total biomass of pure grass swards is underestimated (Figure 4. 3).

Table 4. 3: Relationship between total biomass (g DM m^{-2}) and MPLS and SMLR predictions in experiments 1 and 2 and in the combined dataset.

Analysis	Treatment	N	SEC	R²	SECV	1-VR	Model
Experiment 1							
MPLS	Common	78	6.1	0.99	12.0	0.95	Y=2+x
	RG	29	4.1	0.99	8.9	0.96	Y=1.7+0.96x
	WG	16	1.1	0.99	6.8	0.98	Y=0.6+0.99x
	LG	15	1.5	0.99	9.9	0.96	Y=1.1+0.98x
SMLR	Common	80	10.1	0.97	*	*	Y=2.3+0.97x
Experiment 2							
MPLS	Common	179	12.2	0.93	19.3	0.82	Y=3.7+0.96x
	RG	48	4.3	0.99	21.9	0.76	Y=(-1.8)+x
	WG	46	7.0	0.97	28.0	0.59	Y=(-5.2)+1.1x
	LG	45	14.8	0.88	24.6	0.68	Y=9.3+0.91x
SMLR	Common	188	19.0	0.85	*	*	Y=17.2+0.84
Combined dataset							
MPLS	Common	256	13.0	0.94	19.7	0.85	Y=3.3+0.96x
SMLR	Common	268	22.5	0.83	*	*	Y=16.3+0.83x

MPLS: modified partial least square regression; SMLR: stepwise multiple linear regression; RG: red clover-grass; WG: white clover-grass; LG: Lucerne-grass; N: number of observations; SEC: standard error of calibration; R²: coefficient of determination; SECV: standard error of cross validation; 1-VR: coefficient of determination of cross validation; DM: dry matter; *: not available.

As legume and grass proportions are predicted with similar accuracy by the VIs, only results for EVI are given (Table 4. 6, Figure 4. 4). Apart from the 49 day old swards, there are significant linear relationships between EVI and the proportion of grass and legumes. An increase in legume content and a decrease in grass content generally correspond with an increase in EVI (Figure 4. 4). However, these relations are found only if regression analysis is done separately for the different levels of sward age. The fact, that similar proportions of grass or legumes at different sward ages are connected with very different EVI values, indicates that biomass affects the relationship between spectral signature and species proportion in swards.

4.3.2 Experiment 2

Compared to the swards of experiment 1, phenology of the grass varied much more (from tillering, BBCH 25, to start of flowering, BBCH 61), depending on the length of time of growth after the initial clearing cut to reduce weeds. By contrast, legumes were less developed and remained in the early stages of stem elongation (BBCH 21 to BBCH 31) for all treatments. Proportions of legumes in the mixtures were small with a maximum value of 45%; the grass dominated with proportions up to 99% (Table 4. 2). Weed proportions in the mixtures were small and in the pure grass swards they were <7%, but they increased to 62% in pure legume swards. Total biomass varied from 25 to 296 g m^{-2}. Pure legume swards at advanced stages of growth had almost closed canopies with little soil exposed (<5%, as estimated from digital photographs). By contrast, swards containing ryegrass still had areas of visible soil, even if the biomass yield was large. Grass tillers had considerable proportions of necrotic leaves in the oldest swards.

Table 4. 4: Wavelengths (nm) selected by SMLR analysis to predict the total biomass (g DM m^{-2}) of swards in experiments 1 and 2 and in the combined dataset.

Experiment 1			Experiment 2			Combined experiments		
W (nm)	F	Coeff.	W (nm)	F	Coeff.	W (nm)	F	Coeff.
403	54.2	-2504.3	476	62.7	-18035.6	1144	88.7	10320.4
747	16.0	-2126.7	513	46.0	22431.9	1216	24.0	-15652.8
929	44.4	-13527.3	526	22.2	-8373.5	1226	25.6	-18527.9
938	19.5	20260.2	948	133.3	-24216.9	1276	7.7	10093.4
993	57.4	-4466.3	978	174.4	31640.6	1284	10.0	10354.9
Intercept		24.9	1006	7.8	5919.9	1389	107.7	10769.8
			1116	22.7	6864.9	1782	113.8	-13184.7
			1179	305.6	-23504.1	1850	29.5	8056.2
			1867	133.1	11632.7	2304	11.9	-2242.7
			2291	29.7	-5130.6	Intercept		94.0
			2389	12.2	-2491.5			
			Intercept		50.3			

SMLR: stepwise multiple linear regression; W: wavelength; F: F-value; Coeff.: Coefficient; DM. dry matter.

Compared to the first experiment, the results of the MPLS analysis of the second experiment for calibration and cross validation are slightly poorer (Table 4. 3, Figure 4. 2). Samples identified as outliers in the calibration are pure grass swards as in experiment 1 or they have a total biomass > 164 g m^{-2}. Legume-specific calibrations improve the accuracy of prediction of MPLS for swards of red and white clover-grass mixtures. However, the standard error of cross validation is larger for all legume-specific calibrations compared to the common calibration. As for experiment 1, the plots of the fit for MPLS models result in slopes close to 1 and intercepts close to 0 (Table 4. 3).

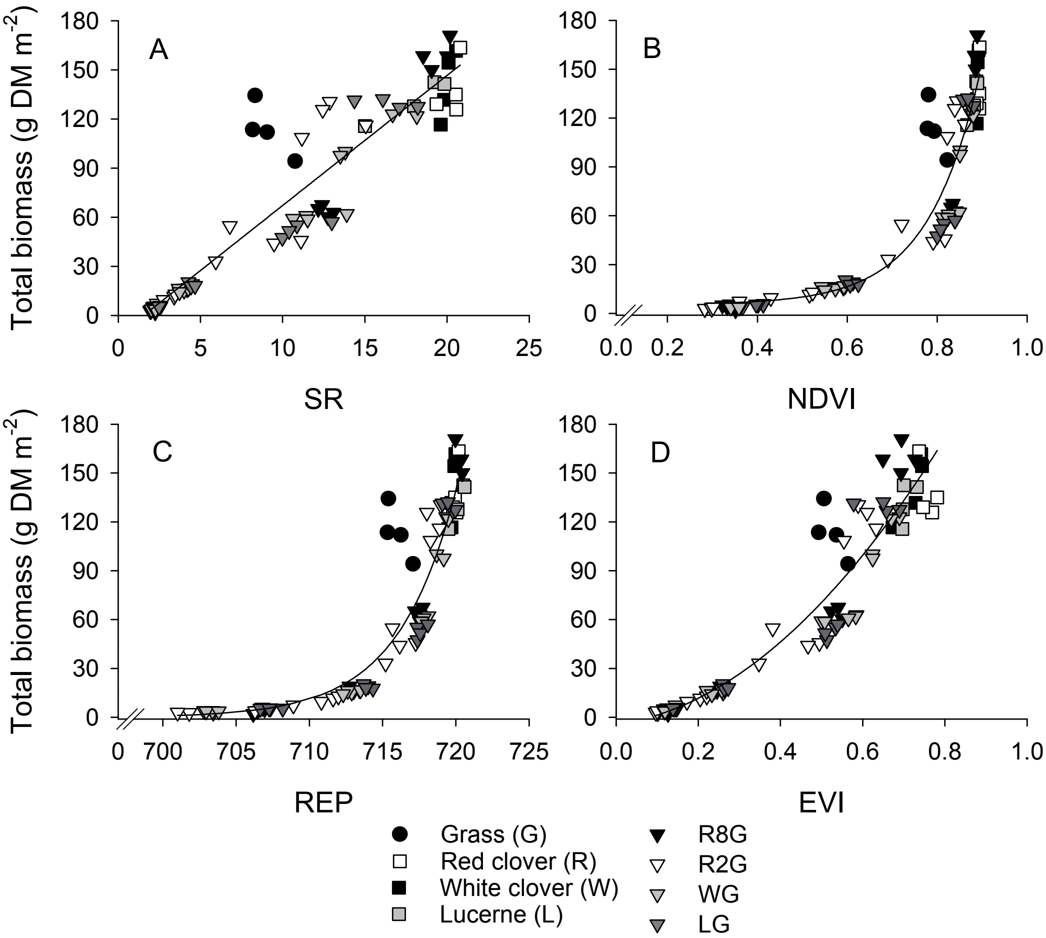

Figure 4. 3: Relationship between total biomass (g DM m^{-2}) and vegetation index: (A) simple ratio (SR), (B) normalized difference vegetation index (NDVI), (C) enhanced vegetation index (EVI) and (D) red edge position (REP) in experiment 1 (statistics of common regressions see Table 4. 5).

Table 4. 5: Relationship between total biomass (g DM m^{-2}) and vegetation index (VI) in experiment 1.

VI	Treatment	N	Regression model	R^2	SE	PSE
SR	Common	80	$y = 8.1\,x - 14.6$	0.89	20.0	
	Grass (G)	4	n.s.			81.7
	Red clover (R)	4	n.s.			23.2
	White clover (W)	4	n.s.			24.0
	Lucerne (L)	4	$y = 5.7\,x + 28.9$	0.94	3.9	7.2
	RG	32	$y = 8.7\,x - 17.2$	0.91	17.2	17.8
	WG	16	$y = 7.4\,x - 14.3$	0.95	10.2	13.1
	LG	16	$y = 8.2\,x - 19.4$	0.90	15.0	15.9
	Mix	64	$y = 8.2\,x - 17.2$	0.91	15.5	15.8
NDVI	Common	80	$y = 1.4 + 0.4\,e^{0.3x}$	0.90	19.4	
	G	4	n.s.			64.0
	R	4	n.s.			22.3
	W	4	n.s.			21.2
	L	4	$y = 1.6\,e^{0.9x}$	0.93	4.1	4.4
	RG	32	$y = 8.2 + 0.003\,e^{12.1x}$	0.91	16.6	18.1
	WG	16	$y = 4.1 + 0.04\,e^{8.9x}$	0.90	13.9	14.5
	LG	16	$y = 10.3 + 0.0003\,e^{14.9x}$	0.94	12.1	15.6
	Mix	64	$y = 9.1 + 0.0009\,e^{13.5x}$	0.92	14.7	16.5
REP	Common	80	$y = 0.9\,e^{0.2(x-700)}$	0.87	20.0	
	G	4	n.s.			92.8
	R	4	n.s.			23.2
	W	4	n.s.			16.5
	L	4	$y = 2.5\,e^{0.2(x-700)}$	0.97	2.5	21.8
	RG	32	$y = 0.5\,e^{0.3(x-700)}$	0.95	12.7	14.0
	WG	16	$y = 0.1\,e^{0.4(x-700)}$	0.98	5.9	12.2
	LG	16	$y = 0.1\,e^{0.4(x-700)}$	0.95	10.9	16.0
	Mix	64	$y = 0.2\,e^{0.3(x-700)}$	0.95	11.8	13.6
EVI	Common	80	$y = 2.1 - 17.3\,x + 294.7\,x^2$	0.90	18.5	
	G	4	n.s.			58.7
	R	4	n.s.			13.7
	W	4	n.s.			36.6
	L	4	n.s.			13.7
	RG	32	$y = 15.2 - 133.1\,x + 477.6\,x^2$	0.93	15.0	17.9
	WG	16	$y = 13.4 - 100.6\,x + 370.6\,x^2$	0.97	8.0	13.5
	LG	16	$y = 24.8 - 183.1\,x + 506.9\,x^2$	0.90	16.0	18.0
	Mix	64	$y = 16.9 - 137.4\,x + 457.5\,x^2$	0.92	14.7	16.2

y: total biomass (g DM m^{-2}); *x*: vegetation index (SR: simple ratio; NDVI: normalized difference vegetation index, EVI: enhanced vegetation index; REP: red edge position); N: number of observations; R^2: coefficient of determination; n.s.: not significant; SE: standard error; PSE: SE of treatment, when the common equation was applied; mix: mixtures without pure swards; DM: dry matter.

The accuracy of SMLR is slightly less than for MLPS (Table 4. 3). The number of wavelengths selected is more than in the first experiment, and only bands 948, 978 and 1006 nm cover similar regions of the spectrum. Additional wavelengths at 479, 513 and 526, 1179, 1867, 2291 and 2389 nm were selected in the second experiment; these cover almost the whole range of the measured spectra (Table 4. 4).

Contrary to the first experiment, regression analysis between VIs and dry biomass reveal only weak relationships across all treatments ($R^2 < 0.2$; data not shown). No relationships were identified for the VIs and the legume and grass proportion of dry biomass in the swards. In addition, MPLS and SMLR analyses were done with the combined data from both experiments. The results are comparable to those of experiment 2 showing a slightly larger SEC (Table 4. 3 and 4. 4).

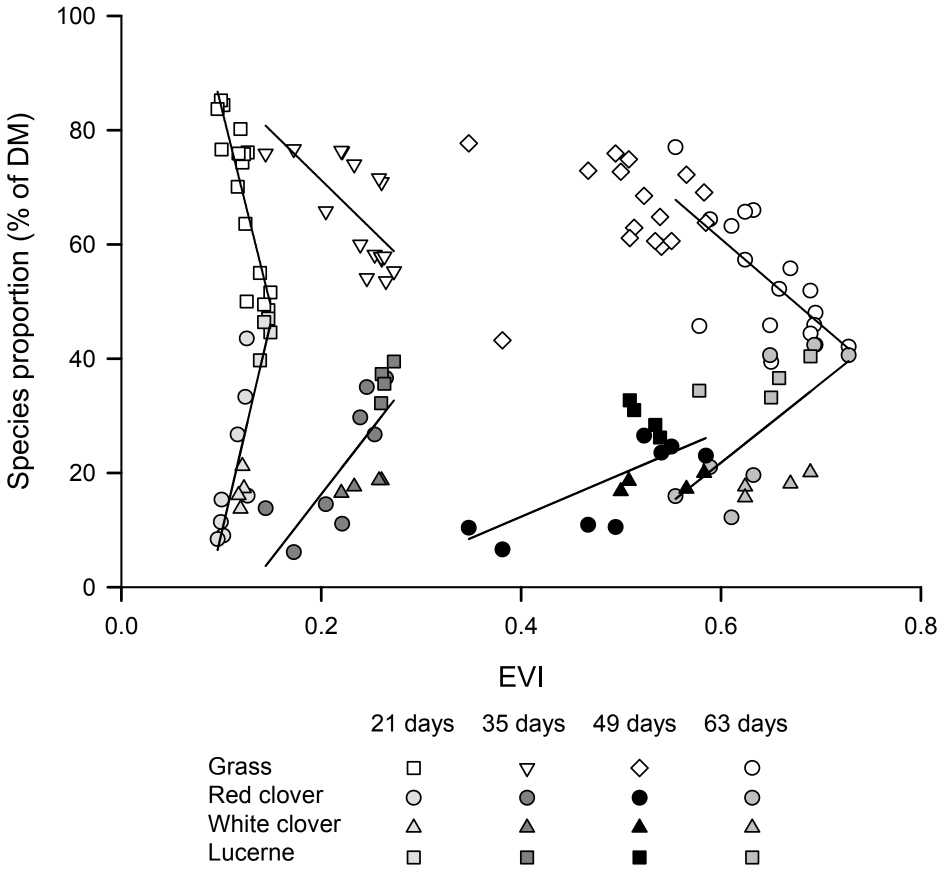

Figure 4. 4: Relationship between grass and legume proportion (% of DM) and enhanced vegetation index (EVI) at different sward ages (21, 35, 49 and 63 days after sowing) in experiment 1 (for regression statistics see Table 4. 6).

Table 4. 6: Relationship between proportion of legumes and grass (% of DM) and enhanced vegetation index (EVI) at different sward age in experiment 1.

DM yield (%)	Sward age	N	Regression model	R^2	SE
Legumes	21	16	$y = -65.42 + 747.33\,x$	0.75	7.4
	35	16	$y = -28.98 + 226.13\,x$	0.56	7.4
	49	16	$y = -17.47 + 74.49\,x$	0.37	6.5
	63	32	$y = -124.02 + 242.42\,x$	0.68	13.0
Grass	21	16	$y = 154.4 - 702.97\,x$	0.75	7.0
	35	16	$y = 105.5 - 171.19\,x$	0.46	6.9
	49	16		n.s.	
	63	32	$y = 264.26 - 343.4\,x$	0.72	16.9

R^2: coefficient of determination; SE: standard error; n.s.: not significant; DM: dry matter

4.4 Discussion

The major objective of the present investigation was to examine the relationship between spectral signatures of legume-grass swards and total biomass across a wide range of legume species, legume proportions and sward age. In both experiments, MPLS analysis achieved the greatest accuracy in predicting total biomass. For the second experiment with more open and mature swards, use of the full range of hyperspectral data resulted in more accurate predictions. This degree of accuracy could not be obtained with VIs, as has been shown in other studies (Cho et al., 2007; Ye et al., 2007). In experiment 1 the plants were still in the vegetative growth stage, whereas in experiment 2 they had generally reached mature growth stages. Moreover, the oldest swards of the second experiment had considerable amounts of dry leaves. Reflectances from swards with larger proportions of grass were also affected by larger areas of visible soil as the grass tiller density was low in the second experiment. Spectral reflectance characteristics change with plant maturation as the fraction of dry biomass increases and the proportion of cell wall material augments in relation to cell contents (Frame, 1992; pp. 146-149). Loss of pigmentation increases visible reflectance, particularly in the red region of the spectrum (Hoffer, 1987). Therefore, with a decline of leaf to stem ratio and plant pigmentation the estimation of total biomass by NDVI (Gamon et al., 1995; Todd et al., 1998) and SR (Starks et al., 2006b) is constrained. This fact illustrates clearly the limits of applying VIs at mature growth stages. However, the problem of varying reflectance characteristics with plant maturation does not necessarily complicate biomass detection in practise. In legume-based forage production systems, swards are usually cut when the grass is at early head emergence. At this growth stage the leaf to stem ratio is still high and the appearance of dead or dry plant material is marginal (Frame, 1992; p. 234). Thus, with sward ages and structures comparable to the conditions in experiment 1 there is scope to obtain useful information with a reduced range of spectral data, as expressed in the VIs.

In relation to the present costs of hyperspectral sensors, it might be preferable to obtain data with only small spectral ranges at the field scale at present. To reduce hyperspectral data to a smaller number of wavelengths and to identify regions of the spectrum that are most important for biomass prediction, we applied SMLR analysis. In the first experiment only three bands were identified; they were in the spectral range partly used to calculate the VIs. The good performance of VIs in the first experiment probably results from the strong correlation between those wavelengths and total biomass. Biomass prediction in the second experiment, however, needed many more bands, as the selected wavelengths were scattered over the whole spectrum. This result supports the fact that VIs have poor accuracy in the prediction of biomass. This suggests that the spectral ranges used for calculating VIs need to be extended with further wavelengths for the sward architectures of the second experiment.

Plant species showed marked differences in canopy structure and reflectance properties, which might result in different VI values at the same level of leaf area index. White clover leaves, for example, are usually arranged in a horizontal plane within the plant canopy, whereas grass leaves are more vertically orientated. In the NIR wavelengths broad-leaved crops always result in larger reflectance values than cereal crops, whereas in the red region they show a similar trend in reflectance (Huete et al., 1997). As the VIs are based on the ratio of NIR and red light, this inevitably leads to larger index values for broad-leaved plants than for cereal crops. This was verified in our study, as legumes always resulted in larger values of the VIs than grass. The results of the present study suggest that the differences in reflectance characteristics only enable the prediction of proportions of grass and legumes when the total biomass of the sward remains constant. With varying levels of biomass, the effect of biomass on the reflectances is superimposed on the relationship between spectral signature and species proportion in the swards. Since biomass affected the reflectances much more strongly than leaf shape and leaf orientation it must be concluded that, for a non-destructive assessment of legume proportion in mixed swards, more appropriate methods, e.g. linear spectral unmixing (Mewes et al., 2008) or other sensors are necessary. Himstedt et al. (2006) showed that digital image analysis gave the most promising results over a wide range of sward age with the same experimental swards. Consequently, with a combination of field spectroscopy and digital image analyses the indirect assessment of legume yield in legume-grass mixtures as a major predictor for atmospheric nitrogen fixation (Høgh-Jensen et al., 2004) might be possible.

4.5 Conclusion

The following conclusions can be drawn from two greenhouse experiments with pure stands and binary mixtures of different forage legumes and grasses:

i) The MPLS analysis for total biomass resulted in the smallest standard errors of 6 and 12 g m^{-2} in experiments 1 and 2, respectively. The hyperspectral approach, in particular, improved accuracy when the spectral signals were confounded as a consequence of advanced maturity of the crop and/or enhanced signals from bare soil. The wavelengths selected by SMLR analysis were different for the two experiments. Swards with large leaf to stem ratios (experiment 1) resulted in similar wavelengths to those in the spectral range of VIs, whereas wavelengths of swards with small leaf to stem ratios, indicating an increased sward maturity (experiment 2) were scattered over the whole spectrum.

ii) Total biomass in experiment 1 was well predicted by the vegetation indices; EVI proved to be the most appropriate one with the smallest standard errors and good accuracy even at higher biomass levels. Accuracy of prediction was improved further by legume-specific calibrations. Weak relationships only were identified for swards in experiment 2.

iii) The prediction of legume proportion by field spectroscopy is difficult as the species-specific signals are confounded by the effects of biomass.

The results of this study suggest that biomass can be determined from field spectral measurements. Nevertheless, these results were obtained under controlled conditions in a greenhouse. Further research is necessary to prove the potential of the technique at the field scale where signatures might be masked by variable light conditions.

5 Determination of dry matter yield from legume-grass swards by field spectroscopy

Abstract An efficient and accurate detection of dry matter (DM) yield of legume-grass mixtures can facilitate a targeted and site-specific management of legume-based swards. The major objective of this study was to examine the relationship between spectral signatures of legume-grass swards and DM yield across a wide range of legume species (white clover, red clover, lucerne, birdsfoot trefoil), legume proportion (0 to 100% of DM) and growth stage (beginning of tillering to end of flowering). Modified partial least squares (MPLS) regression, stepwise multiple linear regression (SMLR) and the vegetation indices (VIs) simple ratio (SR), normalized difference vegetation index (NDVI), enhanced vegetation index (EVI) and red edge position (REP) were used for analysis of the hyperspectral data set (350-2500 nm). Compared to common calibrations, legume-specific models achieved better results, indicating that each legume species had its own spectral characteristics. MPLS and SMLR gave best R^2 values ranging in cross validation from 0.74 to 0.92 with a standard error below 92 g DM m^{-2}. The DM yield prediction by VIs resulted in unsatisfactory accuracies. Prediction accuracy for MPLS and SMLR models were still acceptable even with a reduced spectral data set (630 to 1000 nm), a finding which could facilitate an application of field spectroscopy in practice.

Keywords: Field spectroscopy • Legume-grass • Modified partial least squares (MPLS) regression • Stepwise multiple linear regression (SMLR) • Vegetation indices.

5.1 Introduction

The ability to fix atmospheric nitrogen is one of the main advantages of legumes as components of swards for forage production (Boller and Nösberger, 1987). In organic agriculture especially, which is usually nitrogen-limited (Watson et al., 2002), legume-grass mixtures are an essential contributor to the nitrogen supply in crop rotations. Legume-grass swards are known for their high spatial and temporal variability, due to disturbances such as cutting, frost, drought damage and lack of nutrients. As a result, the functional and structural ground cover patterns become irregular and gaps in the canopy can occur. A site specific determination of dry matter (DM) yield in the field would aid in detecting and quantifying this heterogeneity and optimize field management. Various non destructive approaches have been investigated to determine biomass of different grassland types based on measuring the reflected light from their canopies. Satellite-derived vegetation indices (VIs) have been widely used to estimate grassland biomass (Boschetti et al., 2007; Numata et al., 2007; Todd et al., 1998). However, VIs are highly site and sensor specific (Huang et al., 2004) and based on infrared to red ratios, saturate around a leaf area index of about 2.0-2.5 (Heege et al., 2008) which limit their applicability at higher biomass levels.

Other techniques widely used in laboratory spectroscopy are the analysis of spectral data with partial least square regression (PLS) (Cho et al., 2007; Gianelle and Guastella, 2007) and stepwise multiple linear regression (SMLR) (Huang et al., 2004; Thenkabail et al., 2000). The advantage of PLS is the inclusion of the whole hyperspectral data range into the analysis, resulting in lower losses of spectral information (Haaland and Thomas, 1988; Nguyen et al., 2006). However, few studies have explored the potential of PLS for estimating vegetation parameters using field or satellite data (Cho et al., 2007; Schmidtlein and Sassin, 2004; Huang et al., 2004). The great potential of PLS, offering the use of the whole hyperspectral range, still remains to be examined for the estimation of DM yield from legume-grass swards.

Nevertheless, for practical implementation at the field scale, hyperspectral measurements are very expensive and therefore the use of a small range of the whole spectrum is desirable. Stepwise multiple linear regression (SMLR) using a defined range of the whole spectrum has been applied in estimating plant biochemical composition and biomass by identifying wavebands related to the constituent of interest (Curran et al., 1992; Nguyen et al., 2006; Park et al., 1997). The SMLR method suffers from the potential of overfitting and the selection of bands that fail to correspond with known absorption bands (Curran et al., 1992; Grossman et al., 1996). Nevertheless, recent research has demonstrated that optimal information to quantify characteristics of different plant species is present in a few specific wavebands (Blackburn et al., 1999; Starks et al., 2008; Thenkabail et al., 2004), where particularly those of the red edge region proved to be important for the estimation of biomass (Cho et al., 2007; Gianelle and Guastella, 2007; Hansen and Schjoerring 2003).

We conducted a two-year study to determine relationships between canopy reflectance and DM yield of different legume-grass swards. The specific objectives were:

i) to determine if the vegetation indices, simple ratio, normalized difference vegetation index, enhanced vegetation index and red edge position, based on signals at specific wavelengths are appropriate indicators to determine DM yield of legume-grass swards.

ii) to develop reflectance algorithms for the prediction of DM yield based on the total reflectance in the visible and near infrared wavelength ranges using modified partial least square regression and stepwise multiple linear regression.

iii) to reduce the hyperspectral data range for DM yield prediction to a few informative bands in order to improve the practical applicability.

5.2 Material and methods

5.2.1 Experimental design and plant sampling

The field experiment was conducted during 2005 and 2006 on the organic experimental farm Neu Eichenberg of the University of Kassel (5123'N, 954'E, 227 m a.s.l.). In addition to pure swards of red clover (*Trifolium pratense* L.), white clover (*Trifolium repens* L.), lucerne (*Medicago sativa* L.) and birdsfoot trefoil (*Lotus corniculatus* L.), binary mixtures of each legume with perennial ryegrass (*Lolium perenne* L.) were tested (Table 5. 1). Pure grass swards were fertilized with five N treatments: 0, 40, 80, 120, 160 kg N ha^{-1} to induce additional growth variation in the first year. The nitrogen was supplied as granulated calcium ammonium nitrate on 28 July 2005. The soil was a sandy loam with 3.6% sand, 73% silt, 23.4% clay and 2% humus. Soil analysis indicated optimum levels of phosphorus, magnesium and potassium and a pH of 6.4. During the two-year experiment the average rainfall was 550 mm and the average temperature 9.9 C.

Table 5. 1: Species, cultivars and seed rates used in the field experiment.

Treatment	Cultivar	Seed rate: legume/grass; kg ha^{-1}	
Perennial ryegrass (G)	Fennema	0/25	
White clover/G	Klondike	4/ 0	4/ 15
Red clover/G	Pirat	8/ 0	8/ 15
Lucerne/G	Ameristand	16/ 0	16/ 15
Birdsfoot trefoil/G	Rocco	8/ 0	8/ 15

The experimental treatments were established in four replicates on 2 June 2005. After a first clearing cut to reduce the growth of weeds on 26 July 2005, the first harvest period with a biweekly sampling interval lasted two months from 26 July to 5 October 2005. In 2006, spring, summer and autumn growth were sampled at weekly intervals to determine effects of growth stages and harvested on 12 June, 25 July and 14 September, respectively. To define plant development the BBCH scale according to Meier (2001) was used. Growth stages are represented by two digits, i.e. germination (01-10), leaf development (11-20), formation of side shoots/tillering (21-30), stem elongation or rosette growth, (31-40), development of harvestable vegetative plant parts or vegetatively propagated organs/booting (41-50), inflorescence emergence/heading (51-60), flowering (61-70) and development of fruit (71-80). Due to frost damage, pure stands and mixtures of lucerne and birdsfoot trefoil were analysed in 2006 only at the harvest dates. Total biomass was determined one day after spectral measurements by cutting herbage at a height of 5 cm above soil surface. Samples were dried at 65 C for 48 h.

5.2.2 Spectral data collection

Spectral measurements were conducted with a FieldSpec Pro JR (Analytical Spectral Devices, CO, USA). This type of field spectrometer measures light energy reflected from swards in the range from 350 to 2500 nm with a spectral resolution of 3 nm (350-1000 nm) and 30 nm (1000-2500 nm). Measurements were then interpolated by the analytical spectral devices (ASD) software RS_3^{TM} to produce readings at an interval of 1 nm. The sensor optic had a field of view of 25, which was stabilized on a tripod at a height of 1.07 m above soil. Where possible, readings were taken on unclouded atmospheric conditions with stable lighting conditions between 10:00 and 14:00 h Central European Time. Depending on light conditions spectral calibrations were carried out at least after every 6th measurement using a Spectralon panel (Labsphere, Inc., North Sutton, NH, USA). Each radiometric data point represented four measurements consisting of 40 replicated scans.

5.2.3 Analysis of spectral data

Prior to spectral analysis, spectra were smoothed using eleven convoluting integers and a polynomial of degree five (Savitzky and Golay, 1964; Erasmi and Dobers, 2004). Subsequently, three types of spectral analysis were conducted to estimate DM yield (g m^{-2}) of swards:

i) Four common vegetation indices were calculated from the spectral dataset, simple ratio (SR), normalized difference vegetation index (NDVI), enhanced vegetation index (EVI) and red edge position (REP), using the following equations:

$$SR = \frac{R_{NIR}}{R_{RED}} \tag{5.1}$$

$$NDVI = \frac{R_{NIR} - R_{RED}}{R_{NIR} + R_{RED}} \tag{5.2}$$

$$EVI = G * \frac{R_{NIR} - R_{RED}}{R_{NIR} + C_1 * R_{RED} - C_2 * R_{BLUE} + L} \tag{5.3}$$

$$REP = 700 + 40 * \frac{\left(\frac{R_{670} + R_{780}}{2}\right) - R_{700}}{R_{740} - R_{700}} \tag{5.4}$$

Where *R* is the reflectance value; *NIR* the range of reflectance from 760 to 900 nm; *Red* the range of reflectance from 630 to 690 nm; *Blue* the range of reflectance from 450 to 520 nm (NIR, red and blue were adapted to the bands widths of the Landsat Thematic Mapper); *L* the canopy background adjustment that addresses nonlinear, differential NIR and red radiant transfer through a canopy (here L=1); C_1 and C_2 the coefficients of the aerosol resistance term, which use the blue light to correct for aerosol influences in the red band (here C_1 = 6, C_2 = 7.5); and *G* the gain factor to limit the EVI value to the -1 to +1 range (here G = 2.5; Huete et al., 2002).

Vegetation indices were subjected to regression analysis to estimate the DM yield via the GLM and NLIN procedures of SAS 9.1 (SAS Institute, 2002-2003).

ii) Modified partial least square (MPLS) regression, which is a method used for data compression by reducing the large number of measured collinear spectral variables to a few non-correlated latent variables or factors (Cho et al., 2007). As in multiple regression, the main purpose of MPLS is to build a linear model:

$$y = b_1 x_1 + b_2 x_2 + \ldots\ldots + b_n x_n + e \tag{5.5}$$

Where *y* refers to the response variable (DM yield in this study), *x* is the predictor variable (here the spectral wavelengths reduced to independent factors), *b* indicates the regression coefficients and *e* the residuals (Geladi and Kowalski, 1986). The MPLS equations were developed with the WinISI III (Infrasoft International, LLC. FOSS, version 1.63) software package. Parameters in the mathematical processing were sought through trial and error to minimize the standard error of cross validation, giving best results with the mathematical treatment 1, 4, 4, 1, which means 1: number of derivative of spectra, 4: extent of data points over which the derivative was to be calculated, 4: the smoothing of points, 1: second smoothing, almost never used and normally set as 1. Only every 4th wavelength was used for the calculation of MPLS in order to reduce the computing time.

iii) Stepwise multiple linear regression (SMLR) was performed to select those wavelengths that are mostly correlated with the reference values and to build an equation with reduced variables compared to the MPLS. To avoid multicollinearity only every 8th wavelength was used for analysis. The addition of wavelengths to the equation was continued until the F value fell below 7 (WinISI III Manual, 2005).

For MPLS and SMLR analysis reflectance values in three ranges (1351 to 1439 nm, 1791 to 2019 and 2351 to 2500 nm) were omitted from analysis because of instrument noise or interaction with high atmospheric moisture absorption. Furthermore, the calibration procedure divided the samples at least into four groups in order to perform a cross validation, followed by predictions for the values of one group based on the calibrations developed from the other groups. Finally, standard error of cross validation (SECV) was calculated from the average of single SECVs. The number of outlier elimination passes was two for both MPLS and SMLR analysis. Outliers were defined as samples with a spectrum out of the average population spectra (H-outliers) or for which the difference between the reference and the predicted value was much larger than the standard error of cross validation (T-outliers). The limits were set to 10 (H-outliers) and 2.5 (T-outliers), respectively, as suggested by Tillmann (2000).

5.3 Results

5.3.1 Sward characteristics

Swards were investigated at various growth stages ranging from tillering (BBCH 23) to finishing of flowering (BBCH 67), DM yield varied from 5 to 1756 g m^{-2}. Spring growth exhibited the highest yields (1756 g m^{-2}), whereas summer (1008 g m^{-2}) and autumn growth (191 and 185 g m^{-2} for the first and second year, respec-

Table 5. 2: Descriptive statistics of dry matter (DM) yield (g m^{-2}), growth stages and proportion of legumes and weeds.

Growth period		Autumn 2005				Spring 2006				Summer 2006				Autumn 2006			
		N	Min.	Max.	Mean	N	Min.	Max.	Mean	N	Min.	Max.	Mean	N	Min.	Max.	Mean
All treatments	DM yield	203	5.4	555.3	190.7	107	116.4	1755.8	499.5	67	6	1007.9	309.4	82	14.9	583.3	184.7
	LP, % of DM		0	100	7.1		0	100	50.2		0	100	76.7		0	100	76.1
	WP, % of DM		0	100	19.2		0	11.1	0.8		2.8	34.7	5.2		0	21.7	1.5
Grass (G): pure swards	DM yield	79	33	465.5	215.5	19	116.4	643.3	362.6	11	6	50.8	30.4	14	14.9	90	39
	WP, % of DM		0	45.6	13.6		0	2.3	0.1		0	34.7	18.4		0	2	0.1
	BBCH (G)		23	29			25	55			23	25			23	26	
White clover (W): pure swards and mixtures	DM yield	31	30.5	398.7	152	38	121.6	878.6	344.6	22	26.4	461.7	236.1	28	92.2	297.2	162.9
	LP, % of DM		0	74.1	32.8		1.5	100	55.1		69.5	100	89		72.8	100	92.9
	WP, % of DM		2.6	100	41		0	4.4	0.6		0	9	2.2		0	16.9	1.5
	BBCH (W)		55	67			25	41			65	76			61	71	
Red clover (R): pure swards and mixtures	DM yield	32	24.5	416.1	165.4	38	135.2	1755.8	632.6	22	118.7	747.9	400.3	28	40.7	444.9	201.9
	LP, % of DM		0	88.4	28		8.3	100	66.6		86	100	95		67.3	100	92.2
	WP, % of DM		11.6	96.6	36.7		0	7	0.9		0	10.3	2.9		0	21.7	2.3
	BBCH (R)		41	65			25	61			45	65			45	67	
Lucerne (L): pure swards and mixtures	DM yield	31	10.8	535.1	146	6	615.1	1077.5	873.8	6	539.9	1007.9	725	6	227	583.3	433.3
	LP, % of DM		0	91.4	18.7		22.9	100	67.5		86	100	95.3		88.4	100	94.8
	WP, % of DM		0.9	97	42.5		0	11.1	2.5		0	4.9	1.8		0	2.5	0.5
	BBCH (L)		42	55			51	55			61	66			58	62	
Birdsfoot trefoil (B): pure swards and mixtures	DM yield	30	5.5	372.3	137.7	6	565.2	822	697.2	6	102	573.1	340.8	6	166.8	466.8	297.3
	LP, % of DM		0	100	15.3		10.8	99.7	46.3		65.1	97.6	86.7		50.3	98.5	81.7
	WP, % of DM		0	100	45.7		0	5.9	1.3		0	7.2	3.2		0	5.9	1.5
	BBCH (B)		61	67			61	66			65	75			61	65	

L: Legume proportion; WP: Weed proportion; BBCH: phenological growth stage according to Meier (2001).

tively) were lower in yield. In summer the growth of grass swards was affected by very dry weather (maximum 51 g m^{-2}), whereas legume species, with their large root system, still achieved high yields, which were at maximum 462 and 1008 g DM m^{-2} for white clover and lucerne, respectively (Table 5. 2).

Due to the late sowing date (02.06.2005) and three weeks of no rain after sowing, sward establishment was sub-optimal. As a result in the first year high amounts of bare soil and weeds (*Chenopodium album* L.*,* *Capsella bursa-pastoris* L.*,* *Thlapsi arvense* L.*,* *Matricaria perforate* Merat.*,* *Lamium amplexicaule* L.*,* *Convolvulus arvensis* L.*,* *Sonchus asper* L.*,* *Myosotis arvensis* L.*,* *Cirsium arvense* L.*,* *Stellaria media* L.*,* *Brassica napus* L.) occurred, which could not be eliminated by a clearing cut. While the perennial ryegrass colonized the bare soil by tillering, the legumes did not provide much ground cover until the end of the vegetation period. Thus, the average proportion of legumes was very low (7 % of DM) in the first year, whereas in 2006 it increased up to 50 % and 77 % of DM in the spring and summer growth, respectively (Table 5. 2).

5.3.2 Vegetation Indices

An initial step in the analysis was to investigate whether it was possible to determine the DM yield with the spectral indices SR, NDVI, EVI and REP. All models derived from the VIs showed poor prediction accuracy. In the common model, which included all treatments, the best calibration was obtained with the index REP, resulting in a coefficient of determination (R^2) of 0.23 and a standard error (SE) of 215 g m^{-2} (Table 5. 3). However, in practise legume-grass mixtures usually include one legume species, which may develop to almost pure legume or pure ryegrass areas in parts of the field. Therefore legume-specific calibrations were developed, which included mixtures and pure swards of perennial ryegrass and of the respective legume species. The legume-specific calibration improved R^2 up to 0.44, but the SE still remained very high, ranging from 155 to 264 g m^{-2}. The R^2 of models derived from pure swards of legumes and legume-grass mixtures further improved R^2 up to 0.83, whereas the SE (78-339 g m^{-2}) could not be reduced (Table 5. 3). Lowest prediction accuracy was found for white clover-grass mixtures, where no significant relationships were found.

Table 5. 3: Regression statistics of measured dry matter yield (g m^{-2}) and the vegetation indices enhanced vegetation index (EVI), normalized difference vegetation index (NDVI), red edge position (REP) and simple ratio (SR).

	EVI				NDVI			REP			SR		
	N	SE	R^2	Model	SE	R^2	Model	SE	R^2	Model	SE	R^2	Model
Common	459	226	0.15	q***	225	0.16	q***	215	0.23	e***	225	0.16	l***
Mixtures including pure legume and grass swards													
White clover (W)	179	159	0.1	q***	155	0.10	q***	157	0.11	e***	160	0.08	l***
Red clover (R)	180	264	0.23	e***	263	0.18	q***	232	0.40	e***	253	0.29	l***
Lucerne (L)	109	203	0.41	q***	207	0.21	q***	204	0.40	e***	197	0.44	l***
Birdsfoot trefoil (B)	108	168	0.32	e***	174	0.13	q***	178	0.23	e***	197	0.22	l***
Pure swards only													
W	59	102	0.12	q*	101	0.12	l**	168	0.32	e***		n.s.	l
R	60	248	0.11	e***	339	0.23	l**	208	0.37	e***	242	0.15	l**
L	25	193	0.67	q***	200	0.64	q***	182	0.69	e***	201	0.62	l***
B	24	107	0.81	q***	155	0.60	q***	98	0.83	e***	170	0.50	l***
Grass (G)	123	155	0.10	q**	153	0.13	q***	159	0.05	e*	161	0.04	l*
Mixtures only													
WG	60		n.s.	q		n.s.	q		n.s.	e		n.s.	l
RG	60	245	0.14	q**	240	0.17	q**	208	0.37	e***	242	0.15	l**
LG	24	236	0.46	q***	225	0.51	q***	216	0.53	e***	213	0.54	l***
BG	24	173	0.31	e*		n.s.	q		n.s.	e		n.s.	l

* Significant at the 0.05 probability level; ** Significant at the 0.01 probability level; *** Significant at the 0.001 probability level; l: linear: y=a+bx; q: quadratic: ($y=a+bx+cx^2$); e: exponential: ($y=ae^{bx}$); SE: standard error.

Table 5. 4: Calibration statistics of the prediction of dry matter yield (g m^{-2}) by modified partial least squares regression including sample number (N), mean and standard deviation (SD) of the calibration data.

		Calibration				Cross validation		
Treatment	N	Mean	SD	SEC	R²	SECV	1-VR	RPD
Common	434	240.2	180.7	71.7	0.84	88.4	0.76	2.0
Mixtures including pure legume and grass								
White clover (W)	170	207.6	153.7	64.5	0.82	72.5	0.78	2.1
Red clover (R)	171	256.3	217.5	74.7	0.88	89.3	0.83	2.4
Lucerne (L)	102	222.1	228.4	56.9	0.94	76.9	0.89	3.0
Birdsfoot trefoil (B)	102	202.6	190.2	50.9	0.93	64.0	0.89	3.0
Pure swards only								
W	59	210.3	106.8	71.1	0.56	83.8	0.39	1.3
R	56	323.0	251.5	107.9	0.82	125.7	0.76	2.0
L	23	280.2	249.8	62.7	0.94	95.4	0.85	2.6
B	23	267.2	240.0	50.3	0.96	63.4	0.93	3.8
Grass (G)	118	201.8	161.2	41.7	0.93	62.7	0.85	2.6
Mixtures only								
WG	58	254.0	188.0	65.7	0.88	89.3	0.78	2.1
RG	59	317.5	240.5	79.3	0.89	109.5	0.80	2.2
LG	24	341.2	307.6	61.1	0.96	132.9	0.81	2.3
BG	22	208.3	161.3	29.7	0.97	68.1	0.83	2.4

SEC: standard error of calibration; SECV: standard error of cross validation;
1- VR: coefficient of determination of cross validation; RPD: ratio of standard deviation of the measured results to standard error of cross validation.

5.3.3 Hyperspectral analysis of full spectral data

The MPLS regression predicted DM yield of the common data set with a R^2 of 0.84 and a standard error of calibration (SEC) of 72 g DM m^{-2}. Results of cross validation showed a coefficient of determination of cross validation (1-VR) of 0.76 and a standard error of cross validation (SECV) of 88 g m^{-2}. The residual predictive value (RPD) was 2.0 (Table 5. 4), which represents the standard deviation of the field data divided by the standard error of cross validation and provides a comparison of the performance of all calibrations irrespective of the units of the investigated parameters (Park et al., 1997). An RPD value greater than three is considered adequate for analytical purposes in most of the laboratory near infrared applications for agricultural products (Cozzolino et al., 2006). However, at field scale variable measurement conditions reduce prediction accuracy, so that somewhat lower RPD values may indicate good results. According to Therhoeven-Urselmans et al. (2006) satisfactory prediction results are given in laboratory for

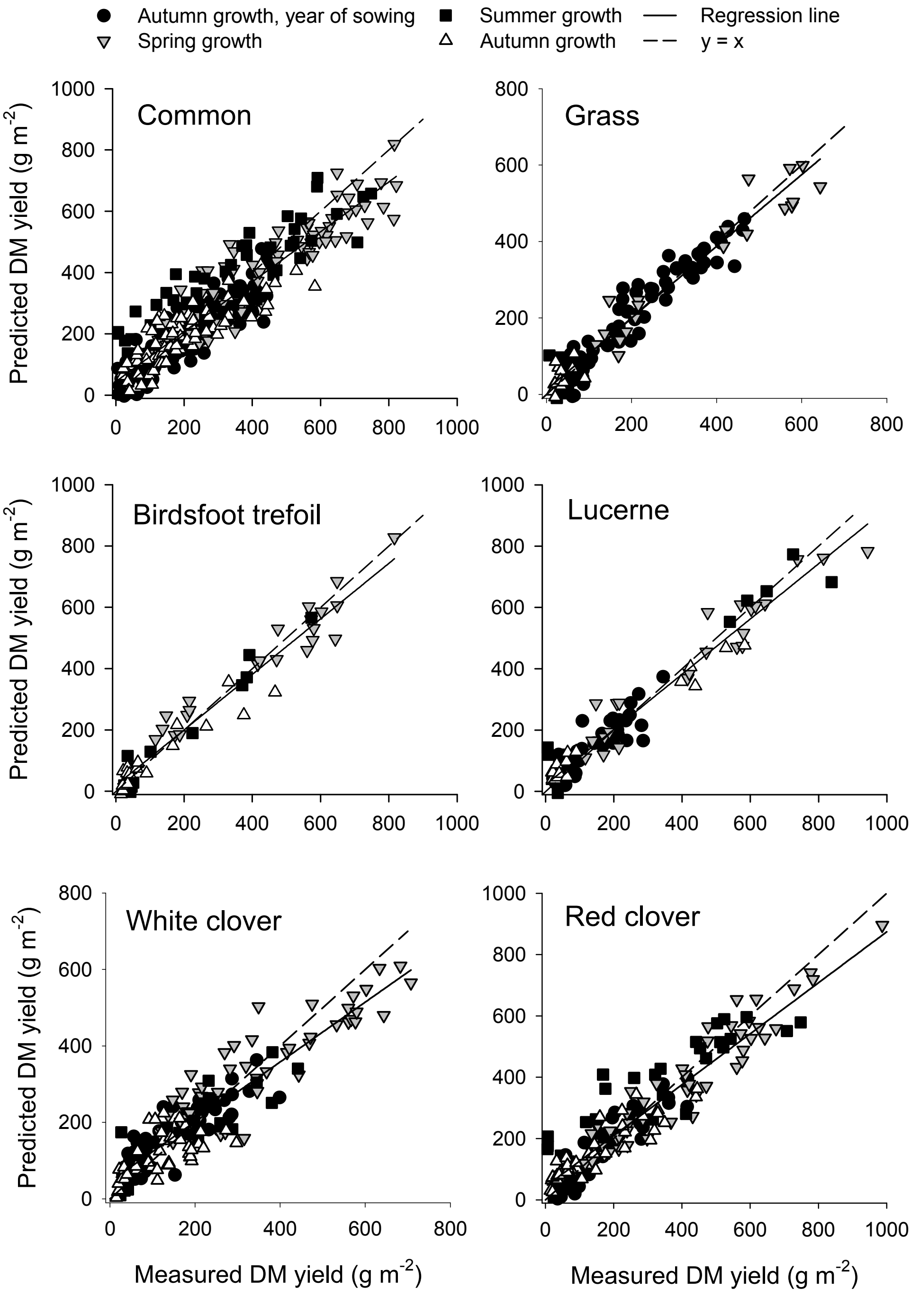

Figure 5. 1: Relationship between the MPLS-predicted and the measured dry matter yield for the common, pure grass and legume-specific calibrations (legume-specific calibrations are composed by binary legume-grass mixtures, pure legume and pure grass swards; for regression statistics see Table 5.4).

organic matter in soil and litter if 1.4 ≤ RPD ≤ 2.0 and good results, if RPD is higher than 2.0. In the calibration procedure 25 outliers were eliminated, which were mainly samples from mature swards (BBCH 55-76). Legume-specific calibrations resulted in an improved prediction accuracy for all models, especially for lucerne and birdsfoot trefoil (Figure 5. 1, Table 5. 4). Similar to the common model, samples in legume-specific calibrations were detected as ouliers if their DM yield exceeded either 900 g m^{-2} or was very low (6-7 g m^{-2}).

Calibrations for pure swards of white clover showed the least accuracy (R: 0.56; 1-VR: 0.39); however, if white clover was combined with grass in the mixture, prediction accuracy improved (R: 0.88; 1-VR: 0.78). Good prediction results were found for all other species, both grown in pure swards as well as in mixtures (Table 5. 4). Covariance analysis was used to assess the effect of growth period (autumn growth in the year of sowing, spring, summer and autumn growth in the following year) on the prediction accuracy of legume-specific models (data not shown). Only for the pure grass a significant interaction between the growth period and the predicted DM could be detected, which legitimises the inclusion of all periods within one legume-specific model.

Prediction accuracy of SMLR calibrations was slightly lower than that of MPLS regression, (Table 5. 5). The common equation achieved a R^2 of 0.74 with a SEC of 93 g DM m^{-2}. Cross validation achieved similar results with 1-VR of 0.74 and SECV of 94 g DM m^{-2}. Similar to the MPLS models, legume-specific calibrations resulted in an improved prediction accuracy, irrespective of the legume species. In contrast to MPLS, the SMLR procedure detected fewer outliers, usually among the group of mature swards, and wavebands selected were scattered over the whole spectrum and differed widely among the legume species. Remarkably, SMLR of pure legume swards resulted in a maximum of 5 significant wavebands, whereas for pure grass swards 9 wavebands were included. Across legume species, the number of significant wavebands in the models parallels the proportion of grass in the sward, with a maximum of 5 wavebands for pure legume swards and 9 wavebands for pure grass swards, legume-grass mixtures being intermittent. Generally, the red (620-750 nm) and short wave near infrared (750-1100 nm)

Table 5. 5: Wavebands (nm) selected for the prediction of dry matter yield (g m^{-2}) by stepwise multiple linear regression and its calibration statistics, including sample number (N), mean, and standard deviation (SD) of the calibration data.

			Calibration				Cross validation		
Treatment	Selected wavebands	N	Mean	SD	SEC	R^2	SECV	1-VR	RPD
Common	359, 479, 847, 855, 887, 947, 1339, 1732, 1740	436	241.2	183.8	92.9	0.74	93.9	0.74	1.9
Mixtures including pure legume and grass swards									
White clover (W)	555, 659, 851, 875, 1019, 1043, 1584	170	214.2	165.9	65.3	0.85	67.3	0.83	2.5
Red clover (R)	363, 859, 1003, 1019, 1131, 1307, 1331	172	260.4	223.4	89.5	0.84	92.7	0.83	2.4
Lucerne (L)	379, 763, 971, 1035, 1299, 1496, 1728, 1736	106	239.5	252.3	82.3	0.89	86.6	0.88	2.9
Birdsfoot trefoil (B)	643, 843, 1043, 1544, 1712	105	206.3	197.9	59.2	0.91	61.8	0.90	3.2
Pure swards only									
W	691, 1003, 1688, 1728, 1744	57	207.3	102.7	50.0	0.76	53.6	0.72	1.9
R	1019, 1331, 1720	55	310.8	236.4	99.7	0.82	105.6	0.80	2.2
L	1011, 1067, 1235	25	341.2	319.2	101.4	0.90	111.7	0.87	2.9
B	1059, 2292	24	265.2	234.9	54.2	0.95	60.5	0.93	3.9
Grass (G)	387, 419, 699, 843, 955, 1339, 1584, 1712, 1752	119	204.6	161.4	51.2	0.90	53.9	0.89	3.0
Mixtures only									
WG	699, 803, 1059, 1616	57	248.5	168.8	50.4	0.91	52.3	0.90	3.2
RG	1003, 1019, 1315, 1688	59	333.7	261.6	91.9	0.88	96.7	0.86	2.7
LG	1011, 1664, 2300	24	341.2	307.6	111.0	0.87	126.3	0.82	2.4
BG	1051, 1219, 2100	24	240.7	200.5	60.4	0.91	72.8	0.86	2.8

SEC: standard error of calibration; SECV: standard error of cross validation; 1- VR: coefficient of determination of cross-validation; RPD: ratio of standard deviation of the measured results to standard error of cross validation; Underlined numbers are indicating the two most important wavelengths for DM yield prediction.

wavebands had the highest F-values, indicating their importance for DM yield prediction.

5.3.4 Hyperspectral analysis of reduced spectral data

Due to the importance of red and short wave near infrared wavebands in DM yield detection as indicated by SMLR, the hyperspectral data were reduced to a range of 620 to 1000 nm with a resolution of 10 nm. This range is adapted to the Yara N-sensor® (FS; Yara International ASA, Oslo, Norway) which is already used for site-specific fertilizer applications in practise.

In comparison to the full data set the reduction resulted in lower prediction accuracy for MPLS models, except for pure white clover swards and white clover-grass mixtures (Table 5. 6). However, 9 out of 15 models showed RPD>2.0, indicating good prediction accuracy. Similar to the analysis of the hyperspectral data range, samples were eliminated as outlier (20 for the common model), either if the DM yield was very high (> 816 g m^{-2}) or if they were in advanced growth stages (BBCH stages 55-76). Prediction accuracy of SMLR analysis was similar to that of MPLS (Table 5. 7), but fewer wavebands were detected as being important in the model. Again, legume specific calibration improved model accuracy leading to a RPD higher than 2.0.

5.4 Discussion

The main objective of our study was to examine the relationship between spectral signatures of legume-grass swards and DM yield across a wide range of legume species, legume proportion and growth stage. Analysis of the full hyperspectral data range by MPLS and SMLR resulted in the highest accuracy for DM yield prediction. Compared to common calibrations, legume specific models achieved better results, indicating that each legume species had its own spectral characteristics. However, DM yield of pure white clover swards was difficult to determine.

One reason may be the lower variability in DM yield compared to the other legumes. Another reason may be the structure of white clover swards, characterized by a dense layer of horizontally oriented clover leaves at a height of 10 to 20 cm

Table 5. 6: Calibration statistics of the prediction of dry matter yield (g m^{-2}) by modified partial least square regression with the reduced data set (620 to 1000 nm, resolution 10 nm) including sample number (N), mean of the calibration data and standard deviation (SD).

	Calibration					Cross validation		
Treatment	N	Mean	SD	SEC	R²	SECV	1-VR	RPD
Common	439	240.3	183.6	110.6	0.63	114.2	0.61	1.6
Mixtures including pure legume and grass swards								
White clover (W)	174	209.4	159.0	83.1	0.73	89.8	0.69	1.8
Red clover (R)	172	259.0	223.1	108.2	0.76	116.2	0.73	1.9
Lucerne (L)	103	220.1	224.9	85.0	0.86	94.8	0.82	2.4
Birdsfoot trefoil (B)	101	196.0	180.9	67.3	0.86	76.1	0.83	2.4
Pure swards only								
W	57	211.8	108.1	61.6	0.68	83.7	0.4	1.3
R	56	323.0	251.5	129.8	0.73	150.3	0.66	1.7
L	23	280.0	249.8	90.6	0.87	107.0	0.82	2.3
B	24	265.2	234.9	83.4	0.87	93.3	0.85	2.5
Grass (G)	116	197.1	157.1	66.0	0.82	73.2	0.79	2.1
Mixtures only								
WG	56	254.2	178.9	49.7	0.92	63.9	0.88	2.8
RG	60	330.9	260.2	98.2	0.86	124.2	0.78	2.1
LG	23	310.2	273.6	110	0.84	155	0.69	1.8
BG	21	202.9	163.2	43.3	0.93	70.2	0.82	2.3

SEC: standard error of calibration; SECV: standard error of cross validation; 1- VR: coefficient of determination of cross validation; RPD: ratio of standard deviation of the measured results to standard error of cross validation.

(Sanderson et al., 2006), irrespective of the clover's growth stage. Regularly dispersed leaves of white clover in the top layers causes a high effective light extinction coefficient (Lantinga et al., 1999) resulting in a strong but undifferentiated pattern of absorption and reflection of light and thus contributing to the weak relationship between DM yield and spectral signature. Growth stage influenced the reflectance characteristics and model development both with SMLR and MPLS. For example, plants at advanced growth stages (especially flowering plants) exhibited spectral attributes that differed strongly from those of less mature swards, so that some of them were eliminated as outliers. However, this should not prevent the use of the technology in practice, as legume-based swards are commonly grown as short-term leys with a limited species diversity and usually are cut when grasses are at early head emergence and legumes are in the early stage of flowering (Frame, 1992), stages which were well represented by the range of phenology of the swards used in this study.

Table 5. 7: Wavebands (nm) selected for the prediction of dry matter yield (g m^{-2}) by stepwise multiple linear regression with the reduced data set (620 to 1000 nm, resolution 10 nm) and its calibration statistics, including sample number (N), mean, and standard deviation (SD) of the calibration data.

			Calibration				Cross validation		
Treatment	Selected wavebands	N	Mean	SD	SEC	R^2	SECV	1-VR	RPD
Common	670, 710, 750, 860, 890, 950	439	240.4	183.0	110.5	0.64	111.7	0.63	1.6
Mixtures including pure legume and grass swards									
White clover (W)	750, 820, 920, 940, 950, *970*	171	208.0	159.6	69.6	0.81	71.2	0.80	2.2
Red clover (R)	730, 740, 770, 840, 880, 980, 1000	171	266.3	233.4	83.3	0.87	86.1	0.86	2.7
Lucerne (L)	620, 640, 740, 850, 880, 990	103	230.2	238.4	83.9	0.88	88.0	0.86	2.7
Birdsfoot trefoil (B)	730, 840, 890, 1000	107	202.6	197.9	84.5	0.82	88.0	0.80	2.3
Pure swards only									
W	620, 730, 740, 830, 880	58	211.7	107.1	67.3	0.61	71.0	0.55	1.5
R	650, 670, 740, 750, 850	56	346.0	294.4	121.0	0.83	127.8	0.81	2.3
L	840, 950	25	341.2	319.2	135.8	0.82	145.9	0.78	2.2
B	950.0	24	265.2	234.9	76.0	0.90	81.7	0.87	2.9
Grass (G)	720, 780, 880, 1000	116	191.7	151.8	67.8	0.80	70.1	0.78	2.2
Mixtures only									
WG	750, 790, 850, 860, 950	58	258.0	187.9	65.4	0.88	70.1	0.86	2.7
RG	670, 730, 860, 890, 950	57	308.8	228.2	90.3	0.84	94.1	0.83	2.4
LG	1000.0	24	341.2	307.6	166.8	0.71	175.9	0.66	1.7
BG	950.0	23	235.0	203.0	94.8	0.78	103.0	0.73	2.0

SEC: standard error of calibration; SECV: standard error of cross validation; 1- VR: coefficient of determination of cross-validation; RPD: ratio of standard deviation of the measured results to standard error of cross validation; Underlined numbers are indicating the two most important wavelengths for DM yield prediction.

Waveband selection by SMLR differed strongly among legume species, which reveals the high sensitivity of SMLR to the initial choice of sward composition for calibration, which is confirmed by other studies (Thenkabail et al., 2000; Huang et al., 2004). Yet Huang et al. (2004) concluded that SMLR appeared to give stable results. In our study, the reliability of SMLR was proved by the evidence, that in nearly all models wavebands of the red and especially the near infrared region were selected as the most important for DM yield prediction which is confirmed by other authors for other crops like rice (Nguyen et al., 2006) cotton, corn, potato and soybean (Thenkabail et al., 2000).

Although SMLR analysis emphasized the high predictive power of the red and near infrared wavelengths, VIs based on these spectral regions showed only poor relationships to DM yield. The heterogeneous plant structure caused by the high proportion of weeds and bare soil in the experimental swards in the first year and the wide range of developmental stages may have confounded the relationships between VIs and DM yield. The reduction of spectral information to only two or three spectral regions probably does not suffice to cover the high variability within the investigated swards for DM yield prediction which is confirmed by other studies (Blackburn et al., 1999; Gamon et al., 1995; Zwiggelaar, 1998). However, the utilization of several narrow wavebands by SMLR and MPLS analysis in the red and near infrared demonstrated the possibility to obtain satisfactory prediction results with both regions. Obviously there is a need to use the information of several specific wavebands instead of averaging the information of the red and near infrared which was done in this study to calculate VIs as used by the broadbands of Landsat Thematic Mapper. This finding is affirmed by many other studies, which found spectral information of narrow-bands to be superior to broad-bands (Blackburn, 1998; Hansen and Schjoerring, 2003; Heege et al., 2008).

In a greenhouse study with similar legume-grass swards, where spectral recordings were taken under controlled conditions with artificial light sources, prediction accuracy for DM yield by MPLS, SMLR and VIs was higher (Biewer et al., 2008). These results indicate the potentials for further improvement of DM yield prediction even under field conditions, which could be achieved e.g. by the use of an additional sensor that measures the incoming radiation to adjust the reflectance signal to changing light conditions or by the application of artificial light to obtain stable measurements which are free of weather interferences.

We think that the direct assessment of essential traits of mixed forage swards in the field would be a major advance in the efficient and environment-friendly management of legume-based farming systems. In parallel studies with the same legume-grass swards the detection of legume proportions by digital image analysis proved promising with a R^2 of 0.7 (Himstedt et al., 2006). Thus, a synchronized determination of total yield and legume proportion by appropriate sensors would allow a more accurate prediction of the nitrogen supply for the succeeding arable crop and help adjust the fertilizer application, as it is well-known that the total annual legume yield is related to the amount of nitrogen fixed during the ley period (Høgh-Jensen et al., 2004; Loges, 1998).

5.5 Conclusions

The vegetation indices, SR, NDVI, EVI and REP, based on signals at specific wavelengths had weak relationships with DM yield. Hyperspectral analysis by MPLS and SMLR resulted in the highest accuracy for DM yield estimation with a standard error of cross validation of 88 and 94 g m^{-2} for MPLS and SMLR, respectively. Accuracy of prediction was improved further by legume-specific calibrations. Although selected wavelengths by SMLR analysis differed for each calibration, in nearly all models the red and especially the near infrared region revealed the highest information for DM yield prediction. The reduction of the hyperspectral data set to the range of 620 to 1000 nm with a resolution of 10 nm gave satisfactory prediction results for both the MPLS and SMLR analysis. Prediction accuracy could be improved if legume-specific calibrations were calculated.

6 Determination of forage quality in legume-grass mixtures using field spectroscopy

Abstract Timely assessments of nutritive values of legume-based swards during the growing season can facilitate a targeted and site-specific forage management. This study was undertaken to explore the potential of field spectral measurements for a non destructive prediction of metabolizable energy (ME), ash content, crude protein (CP) and acid detergent fiber (ADF) of legume-grass mixtures. A population of 200 legume-grass swards [*Lolium perenne* (L.), *Trifolium repens (*L.), *Trifolium pratense* (L.)] representing a wide range of legume proportion (0 to 100% of DM) and growth stages (beginning of tillering to end of flowering) were used in this investigation. The paper examines three techniques for analysis of the hyperspectral data set (350-2500 nm): two-waveband reflectance ratios, modified partial least squares (MPLS) regression and stepwise multiple linear regression (SMLR). Forage quality variables had weak relationships with the developed reflectance ratios, whereas hyperspectral analysis by MPLS and SMLR resulted in high prediction accuracy ($0.70 \leq R^2 \leq 0.94$). Even with a reduced spectral data set (630 to 1000 nm) estimates of MPLS and SMLR models were still acceptable for forage ash ($0.62 \leq R^2 \leq 0.78$) and CP ($0.83 \leq R^2 \leq 0.86$), a finding which could facilitate an application of field spectroscopy in practice. Prediction accuracy for ash and CP was further improved by legume-specific calibrations.

Keywords: Metabolizable energy (ME) • Ash • Crude protein (CP) • Acid detergent fiber (ADF) • Legume-grass • Modified partial least squares (MPLS) regression • Stepwise multiple linear regression (SMLR) • Two-waveband reflectance ratio.

6.1 Introduction

Accurate information on nutritive values of legume-grass swards is extremely useful in livestock and forage management. However, nutritive values of legume-grass swards can vary considerably within a field and during the growing period, due to disturbances such as lack of nutrients, frost, drought damage or defoliation. Hence, a site specific determination of the nutritive values such as metabolizable energy (ME) ash content, crude protein (CP) and acid-detergent fibre (ADF) in the field would help in detecting and quantifying this heterogeneity and optimize field and forage management.

Various non-destructive approaches have been investigated in field crops and scrublands to determine plant biochemical properties and nutrient status by measuring reflectance of the incident light on the leaf (Curran et al., 1992; Zhao et al., 2005) canopy (Hansen and Schjoerring, 2003; Nguyen et al., 2006; Thenkabail et al., 2000), or landscape level (Blackburn and Steele, 1999; Serrano et al., 2002). However, similar studies on the estimation of forage quality variables are limited and basically focused on nitrogen concentration (Gianelle and Guastella, 2007; Lamb et al., 2002; Mutanga et al., 2003).

The determination of forage neutral detergent fibre (NDF), ADF and CP with canopy reflectance of bermudagrass [*Cynodon dactylon* (L.)] pastures were investigated by Starks et al. (2006b) using the broadband vegetation indices (VIs) normalized difference vegetation index (NDVI) and simple ratio (SR). They concluded that these VIs could only explain a small portion of variance in the forage quality variables, whereas further investigations indicated that the development of narrow two-waveband reflectance ratios performed better (Starks et al., 2006a). Also Biewer et al. (2008) found that estimates by broadband VIs (SR, NDVI and enhanced vegetation index) were poor for dry matter yield detection of legume-grass swards, if sward age was beyond heading. Hence, the development of two-waveband reflectance ratios may be an alternative to the broadband VIs for predicting forage nutritive values.

Other techniques for analysing spectral data are modified partial least square regression (MPLS) and stepwise multiple linear regression (SMLR). The advantage of MPLS is the inclusion of the whole hyperspectral data range into the analysis, resulting in lower losses of spectral information (Haaland and Thomas, 1988; Nguyen et al., 2006). However, few studies have explored the potential of MPLS for estimating forage quality constituents using field data. Starks et al. (2004) determined forage NDF, ADF and nitrogen concentrations of bermudagrass with MPLS and found that it could explain 63 to 76% of the variability expressed in the reference data. However, the great potential of MPLS, offering the use of the whole hyperspectral range, still remains to be examined for the estimation of forage quality variables from legume-grass swards.

Although MPLS seems to be a powerful method for the analysis of large data sets, it is not practical for livestock managers to predict forage nutritive variables using an expensive, full range spectrometer. An approach to reduce the range could be the analysis of SMLR, as it enables a few wavelengths to be extracted from the full dataset to create a prediction model. Problems known for the SMLR method are the potential of overfitting and waveband selection that fail to correspond with known absorption bands (Curran et al., 1992; Grossman et al., 1996). However, recent research has demonstrated that much information to quantify characteristics of different plant species is available in a few specific wavebands (Blackburn and Steele 1999; Starks et al., 2008; Thenkabail et al., 2004), where particularly those of 2054 and 2172 nm (Kokaly, 2001) and the red edge region (Gianelle and Guastella, 2007; Lamb et al., 2002) proved to be important for the estimation of nitrogen concentration.

In this study we investigated canopy reflectance of different legume-grass swards in order to determine the contents of ME, ash, CP and ADF. The specific objectives were:

i) to determine, if the development of two-waveband reflectance ratios, based on signals at specific wavelengths are appropriate indicators for the estimation of ME, ash, CP and ADF.

ii) to develop reflectance algorithms for the prediction of the forage quality parameters ME, ash, CP and ADF based on total reflectance in the visible and near infrared wavelength ranges using MPLS regression and SMLR.

iii) to reduce the hyperspectral data range for the prediction of ME, ash, CP and ADF to a few informative bands in order to improve the practical applicability.

6.2 Material and methods

6.2.1 Experimental design and plant sampling

The field experiment was conducted during the year 2006 on the organic experimental farm Neu Eichenberg of the University of Kassel (51°23'N, 9°54'E, 227 m a.s.l.). Pure swards of red clover (*Trifolium pratense* L.), white clover (*Trifolium repens* L.) and perennial ryegrass (*Lolium perenne* L.) as well as binary mixtures of each legume with perennial ryegrass were tested (Table 6. 1). The soil was a sandy loam with 3.6% sand, 73% silt, 23.4% clay and 2% humus. Soil analysis indicated optimum levels of phosphorus, magnesium and potassium and a pH-value of 6.4. In the growing period of the experiment the yearly rainfall was 554 mm and the average temperature 9.6° C.

Table 6. 1: Species, cultivars and seed rates used in the field experiment.

Treatment	Cultivar	Seed rate: legume/grass; kg ha^{-1}	
Perennial ryegrass (G)	Fennema	0/25	
White clover/G	Klondike	4/ 0	4/ 15
Red clover/G	Pirat	8/ 0	8/ 15

The experimental treatments with a size of 29 m² were established on 2. June 2005. In the following year 2006 three main cuts were taken on 12. June, 25. July and 14. September. In between these cuts, samples in a weekly interval were harvested to determine effects of growth stages. To define plant development the BBCH scale according to Meier (2001) was used. Growth stages are represented by two digits, i.e. germination (01-10), leaf development (11-20), formation of side shoots/tillering (21-30), stem elongation or rosette growth (31-40), development of harvestable vegetative plant parts or vegetatively propagated organs/booting (41-50), inflorescence emergence/heading (51-60), flowering (61-70) and development of fruit (71-80). One day after spectral measurements biomass was harvested at a height of 5 cm above soil surface and dried at 65 °C for 48 h. Subsequently, samples were ground with a 1 mm sieve in order to determine the nutritive value.

6.2.2 Assessment of reference data

Reflectance spectra of near infrared spectroscopy (NIRS) measurement were obtained using a XDS-spectrometer (Foss NIRSystems, Hillerød, Denmark). The spectrum of a sample was an average of 25 subscans and was recorded as the logarithm of the inverse of the reflectance [log(1/R)]. Quality parameters were determined using calibrations developed by Loges (1998) with standard errors of cross validation (SECV) of 0.3 MJ kg DM^{-1}, 1.1 % DM, 0.9% DM and 2.0 % DM for ME, ash, CP and ADF, respectively. The calculation was done with the WinISI software (version 1.63, Foss NIRSystems/Tecator Infrasoft International, LLC, Silver Spring, MD, USA), using the range between 1100 and 2498 nm.

6.2.3 Spectral data collection

Spectral measurements in the field were conducted with a FieldSpec® Pro JR (Analytical Spectral Devices, CO, USA). This type of field spectrometer measures light energy reflected from swards in the range from 350 to 2500 nm with a spectral resolution of 3 nm (350-1000 nm) and 30 nm (1000-2500 nm). Measurements were then interpolated by the analytical spectral devices (ASD) software RS_3^{TM} to produce readings at an interval of 1 nm. The sensor optic had a field of view of 25°, which was stabilized on a tripod in a height of 1.07 m above soil. Where possible, readings were taken on unclouded atmospheric conditions with stable lighting conditions between 10:00 and 14:00 h Central European Time. Depending on light conditions spectral calibrations were carried out at least after every 6^{th} measurement using a Spectralon® panel (Labsphere, Inc., North Sutton, NH, USA). Each radiometric data point represented a mean of four measurements consisting of 40 replicated scans.

6.2.4 Processing and analysis of spectral data

Prior to spectral analysis, spectra were smoothed using eleven convoluting integers and a polynomial of degree five (Savitzky and Golay, 1964; Erasmi and Dobers, 2004). Then reflectance values in the ranges from 1800 to 1939 nm and 2430 to 2500 nm were omitted from analysis because of instrument noise or interaction with high atmospheric moisture absorption. Subsequently, three types of spectral analysis were conducted to estimate nutritive values of swards:

i) Two-waveband reflectance ratios were calculated in order to find the optimal spectral regions to estimate ME, ash, CP and ADF. Ratios were developed by averaging spectral data over 10 nm to reduce the number of wavebands to 183.

Pearson correlation coefficients were calculated using the CORR procedure of SAS 9.1 (SAS Institute, 2002-2003) in order to select the wavebands with maximum correlation coefficient (R) for ME, ash, CP and ADF. The reflectance values at these selected wavebands were used as the numerators and reflectance values at all other wavebands were used as denominators to calculate reflectance ratios, according to Zhao et al. (2005).

The two-waveband reflectance ratios were then subjected to regression analysis in order to estimate nutritive values using the GLM procedures of SAS 9.1.

ii) Modified partial least square (MPLS) regression was conducted, which is a method employed for data compression by reducing the large number of measured collinear spectral variables to a few non-correlated latent variables or factors (Cho et al., 2007). As in multiple regression, the main purpose of MPLS is to build a linear model:

$$y = b_1 x_1 + b_2 x_2 + ... + b_n x_n + e \qquad (6.1)$$

Where y refers to the response variable (ME, ash, CP and ADF in this study), x is the predictor variable (here the spectral wavelengths reduced to independent factors), b indicates the regression coefficients and e are the residuals (Geladi and Kowalski, 1986).

iii) Stepwise multiple linear regression (SMLR) was performed to select those wavelengths that are mostly correlated with the reference values and to build an equation with reduced variables compared to the MPLS. In order to avoid multi-collinearity only every 8th wavelength was used for analysis. The addition of wavelengths to the equation was continued until the F value fell below 7 (WinISI III Manual, 2005).

Both, MPLS and SMLR equations were developed with the WinISI software. For MPLS and SMLR analysis parameters in the mathematical processing were sought through trial and error in order to minimize the standard error of cross validation, giving best results with weighted multiplicative scatter correction and mathematical treatment of 1, 4, 4, which means 1: number of derivative of spectra, 4: extent of data points over which the derivative was to be calculated and 4: the smoothing of points. Only every 4th wavelength was used for the calculation of MPLS in order to reduce the computing time. Furthermore the WinISI software divided the samples for the calibration procedure at least into four groups in order to perform a cross validation. Cross validation was conducted by a random separation of the data set into four or more groups followed by predictions for the values of one group based on the calibrations developed from the other groups. In turn, predictions were made for all groups and finally averaged. The number of outlier elimination passes was two for both MPLS and SMLR analysis. Outliers were defined as samples with a spectrum out of the population spectra (H-outliers) or for which the difference between the reference and the predicted value was much larger than the standard error of cross validation (T-outliers). The limits were set to 10 (H-outliers) and 2.5 (T-outliers), respectively, as suggested by Tillmann (2000).

6.3 Results and discussion

6.3.1 Sward characteristics and nutritive values

As swards were investigated at various growth stages ranging from tillering (BBCH 23; Meier, 2001) to finishing of flowering (BBCH 67), nutritive values varied widely over the growth period (Table 6. 2). The ME content was highest in spring with 12.5 MJ kg DM^{-1}, whereas in summer, where the growth of grass was strongly affected by very dry weather, lowest values (6.6 MJ kg DM^{-1}) were obtained. Variation in the stage of maturity was highest in spring, which was reflected in ash and CP contents showing a wide range from 4.2 to 14.1 % DM and 3.5 to 33.6 % DM, respectively. Lowest ash and CP values were found for the pure grass swards. Highest ADF values were achieved in the summer period with 34.8 % DM. Thus, the wide range of nutritive values found in our study provided an appropriate data set for the development of reflectance algorithms, as it covered most of the variability reported in literature for white clover (Berado, 1997), red clover (Wachendorf, 1995) and perennial ryegrass (De Boever et al., 1996).

6.3.2 Correlation among forage quality constituents and relationships between narrowband reflectance and nutritive values

In the common data set linear correlation among nutritive values was highest between ash and ME (r=0.79), as well as between ADF and CP (r=-0.55). In contrast, correlation between CP and ash and between ME and ADF revealed only low values (r=0.34 and r=-0.18, respectively). No significant relation could be found between CP and ME and ADF and ash, respectively (Table 6. 3). Similar results were found for the legume-specific data sets of white and red clover, which included mixtures and pure swards of perennial ryegrass and of the respective legume species.

The correlation between nutritive values and sward reflectance including the waveband range between 355 to 2495 nm is presented in Figure 6. 1. Aside from a parallel shift, canopy reflectance of legume-grass swards exhibited almost similar correlation curves for the ash and CP, although the correlation among these quality variables was relatively low. High positive correlation coefficients were obtained at ranges of 355-370, 540-555, 720-1370 and 1680-1700 nm, indicating similar regions in the spectrum which were somehow correlated with both quality parameters. There were spectral regions significantly correlated with the contents of ME and ADF. However, they varied from that of ash and CP and generally had lower correlation coefficients. Altogether, the correlation of standing pasture reflectance in single wavebands maximally reached a coefficient of determination (R^2) of 0.58, which is not satisfactory for practical application.

Table 6. 2: Descriptive statistics of metabolizable energy (ME), ash, crude protein (CP), acid detergent fibre (ADF), and phenological growth stages for the three growth periods spring, summer and autumn.

		Spring					Summer					Autumn				
		N	Mean	SD	Min.	Max.	N	Mean	SD	Min.	Max.	N	Mean	SD	Min.	Max.
Common	ME, MJ kg^{-1} DM^{-1}	78	11.2	0.8	9.7	12.5	52	9.9	0.9	6.6	11.6	70	11.1	0.4	10.0	12.0
	Ash, % DM		10.4	2.2	4.2	14.1		11.8	1.2	8.7	14.3		12.0	0.9	10.1	14.2
	CP, % DM		17.2	8.9	3.5	33.6		19.8	4.3	11.1	30.0		25.0	5.3	12.5	32.3
	ADF, % DM		21.9	5.8	10.6	32.4		24.0	5.1	13.7	34.8		20.5	3.0	14.6	28.4
Pure grass (G)	ME, MJ kg^{-1} DM^{-1}	16	11.3	0.7	10.1	12.4	8	8.6	1.1	6.6	10.0	14	10.8	0.4	10.0	11.3
	Ash, % DM		8.0	2.1	4.2	11.0		10.6	1.1	8.7	12.4		11.0	0.6	10.3	12.4
	CP, % DM		8.3	4.1	3.5	16.4		13.1	1.1	11.1	14.8		15.5	2.2	12.5	19.1
	ADF, % DM		23.1	4.8	17.0	30.9		19.5	3.8	15.6	25.9		23.8	2.2	18.8	28.4
	BBCH (G)				25.0	55.0				23.0	25.0				23.0	26.0
White clover (W): Pure swards and WG mixtures	ME, MJ kg^{-1} DM^{-1}	31	11.6	0.7	9.9	12.5	22	10.2	0.9	8.0	11.6	28	11.3	0.4	10.3	12.0
	Ash, % DM		11.1	2.0	7.1	14.1		12.0	1.2	9.5	13.9		12.4	0.6	11.2	13.5
	CP, % DM		20.7	9.9	6.0	33.6		21.1	3.4	15.0	30.0		27.7	2.1	24.1	32.3
	ADF, % DM		18.9	5.9	10.6	30.9		23.8	4.8	13.7	32.6		18.5	2.7	14.6	25.2
	BBCH (W)				25.0	41.0				65.0	76.0				61.0	71.0
Red clover (R): Pure swards and RG mixtures	ME, MJ kg^{-1} DM^{-1}	31	10.8	0.6	9.7	11.9	22	10.0	0.5	9.0	11.0	28	11.1	0.3	10.4	11.6
	Ash, % DM		10.9	1.4	8.5	13.8		12.1	1.0	10.2	14.3		12.1	1.0	10.1	14.2
	CP, % DM		18.2	6.3	7.2	30.3		21.0	3.7	14.5	27.0		27.0	2.5	22.1	31.4
	ADF, % DM		24.4	4.8	17.1	32.4		25.9	4.9	17.9	34.8		20.8	2.0	15.6	24.3
	BBCH (R)				25.0	61.0				45.0	65.0				45.0	67.0

SD: Standard deviation; DM: Dry matter; BBCH: phenological growth stage according to Meier (2001).

Table 6. 3: Correlation coefficient among nutritive values.

Nutritive values	ME	Ash	CP
Common	MJ kg^{-1} DM^{-1}	% DM	% DM
ME, MJ kg^{-1} DM^{-1}	1		
Ash, % DM	0.79***	1	
CP, % DM	n.s.	0.34***	1
ADF, % DM	-0.18*	n.s.	-0.55***
White clover-specific data set			
ME, MJ kg^{-1} DM^{-1}	1		
Ash, % DM	0.84***	1	
CP, % DM	n.s.	0.61***	1
ADF, % DM	n.s.	n.s.	-0.62***
Red clover-specific data set			
ME, MJ kg^{-1} DM^{-1}	1		
Ash, % DM	0.84***	1	
CP, % DM	n.s.	0.39***	1
ADF, % DM	n.s.	n.s.	-0.43***

An initial step in the analysis was to investigate whether it is possible to determine ME, ash, CP and ADF with two-waveband narrow reflectance ratios (data not shown). Compared to linear correlation of nutritive values with reflectance data, this approach improved the prediction accuracy only for ME and ADF. The best calibration was obtained for CP with the reflectance ratio R_{1210}/R_{1260}, resulting in a R^2 of 0.33 and a standard error (SE) of 6% DM. The lowest prediction accuracy was found for ADF, which did not exceed a R^2 of 0.15. Several studies on different plant species have indicated that two-waveband reflectance ratios of canopies performed quite well in predicting plant variables (Carter and Spiering, 2002; Hansen and Schjoerring, 2003; Heege et al., 2008). Starks et al., (2006a) used two-waveband reflectance ratios to determine CP of bermudagrass pastures, reaching a R^2 of 0.61 and a root mean square error of 1.47. However, in our study prediction accuracy of two-waveband reflectance ratios was rather poor for forage quality variables. Presumably, heterogeneous sward structures caused by a varying legume dry matter contribution and a wide range of developmental stage, as well as variable atmospheric conditions while taking spectral measurements at field may have confounded the relationships between two-waveband reflectance ratios and quality constituents. Hence, the reduction of spectral information to only two spectral wavebands does not suffice to cover the high variability within the investigated swards.

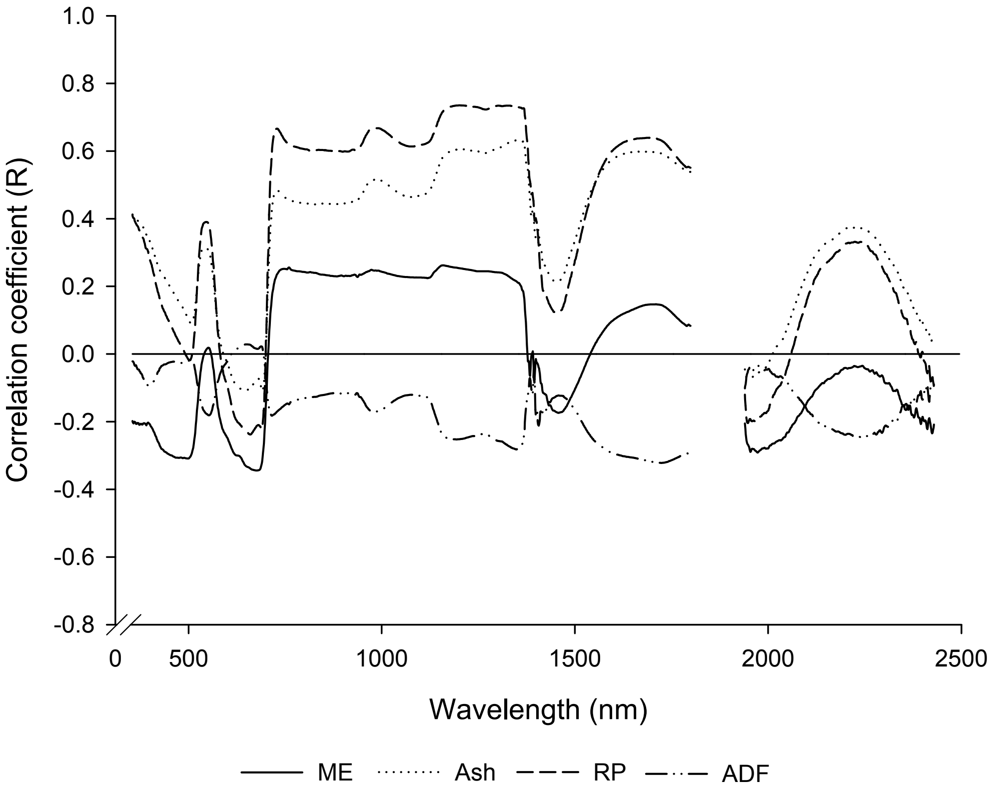

Figure 6. 1: Relationship between canopy reflectance for single wavebands (expressed as Pearson's correlation coefficient) and metabolizable energy (ME), ash, crude protein (CP) and acid detergent fibre (ADF). Wavelengths between 1799 and 1940 nm were omitted from analysis because of interaction with atmospheric moisture absorption.

6.3.3 Hyperspectral analysis of full spectral data

Hyperspectral analysis of the full data set yielded for all constituents in the highest prediction accuracy (Figure 6. 2, Table 6. 4). MPLS calibrations of the common data set explained 80, 87, 93 and 84% of the variance and had standard errors of cross validation (SECV) of 0.4, 0.9, 3.1 and 2.4 for ME, ash, CP and ADF, respectively. Residual predictive values (RPD) ranged between 1.8 and 2.4. RPD represents the standard deviation of the field data divided by the standard error of cross validation and provides a comparison of the performance of all calibrations, irrespective of the units of the investigated parameters (Park et al., 1997). An RPD

Table 6. 4: Calibration statistics of the prediction for metabolizable energy (ME), ash, crude protein (CP) and acid detergent fibre (ADF) by modified partial least squares regression including sample number (N), mean and standard deviation (SD) of the calibration data.

			Calibration				Cross validation		
	Constituent	N	Mean	SD	SEC	R^2	SECV	1-VR	RPD
Common	ME, MJ kg^{-1} DM^{-1}	186	11.0	0.7	0.3	0.80	0.4	0.70	1.8
	Ash, % DM	194	11.4	1.7	0.6	0.87	0.9	0.73	1.9
	CP, % DM	196	20.6	7.6	2.1	0.93	3.1	0.83	2.4
	ADF,% DM	190	22.2	4.8	2.0	0.84	2.4	0.75	2.0
Mixtures including pure legume and grass swards									
White clover	ME, MJ kg^{-1} DM^{-1}	110	11.1	0.8	0.4	0.73	0.5	0.62	1.6
	Ash, % DM	115	11.1	1.9	0.6	0.88	0.8	0.84	2.5
	CP, % DM	113	19.7	8.2	2.1	0.93	2.5	0.90	3.2
	ADF,% DM	116	20.8	4.8	2.7	0.70	3.1	0.59	1.6
Red clover	ME, MJ kg^{-1} DM^{-1}	114	10.7	0.8	0.3	0.81	0.5	0.64	1.7
	Ash, % DM	114	11.1	1.7	0.8	0.78	0.9	0.76	2.0
	CP, % DM	118	18.7	7.2	2.8	0.85	3.0	0.83	2.4
	ADF,% DM	117	23.3	4.4	1.7	0.85	2.3	0.73	1.9

SEC, standard error of calibration; SECV, standard error of cross validation; 1- VR, coefficient of determination of cross validation; RPD, ratio of standard deviation of the measured results to standard error of cross validation.

value greater than three is considered adequate for analytical purposes in most of the laboratory near infrared applications for agricultural products (Cozzolino et al., 2006). However, at field scale variable measurement conditions reduce prediction accuracy, so that even lower RPD values may indicate good results. According to Therhoeven-Urselmans et al. (2006), satisfactory prediction results are given in laboratory for organic matter in soil and litter if $1.4 \leq \text{RPD} \leq 2.0$ and good results, if RPD is higher than 2.0.

Overall model accuracy was lowest for ME and highest for CP, which is also reflected by the number of outliers being excluded from the model. For ME, 14 samples were detected as outlier, which were mainly grass swards of the summer growth with low ME values (< 9 MJ kg DM^{-1}). In summer the growth of grass swards was heavily affected by very dry weather resulting in considerable amounts of dry leaves and larger areas of visible soil which impacts spectral reflectance characteristics (Elvidge and Chen, 1995; Gamon et al., 1995; Todd et al., 1998). Ten outliers were detected in the ADF model predominantly represented by white clover swards at advanced growth stages (BBCH 41-65) or with

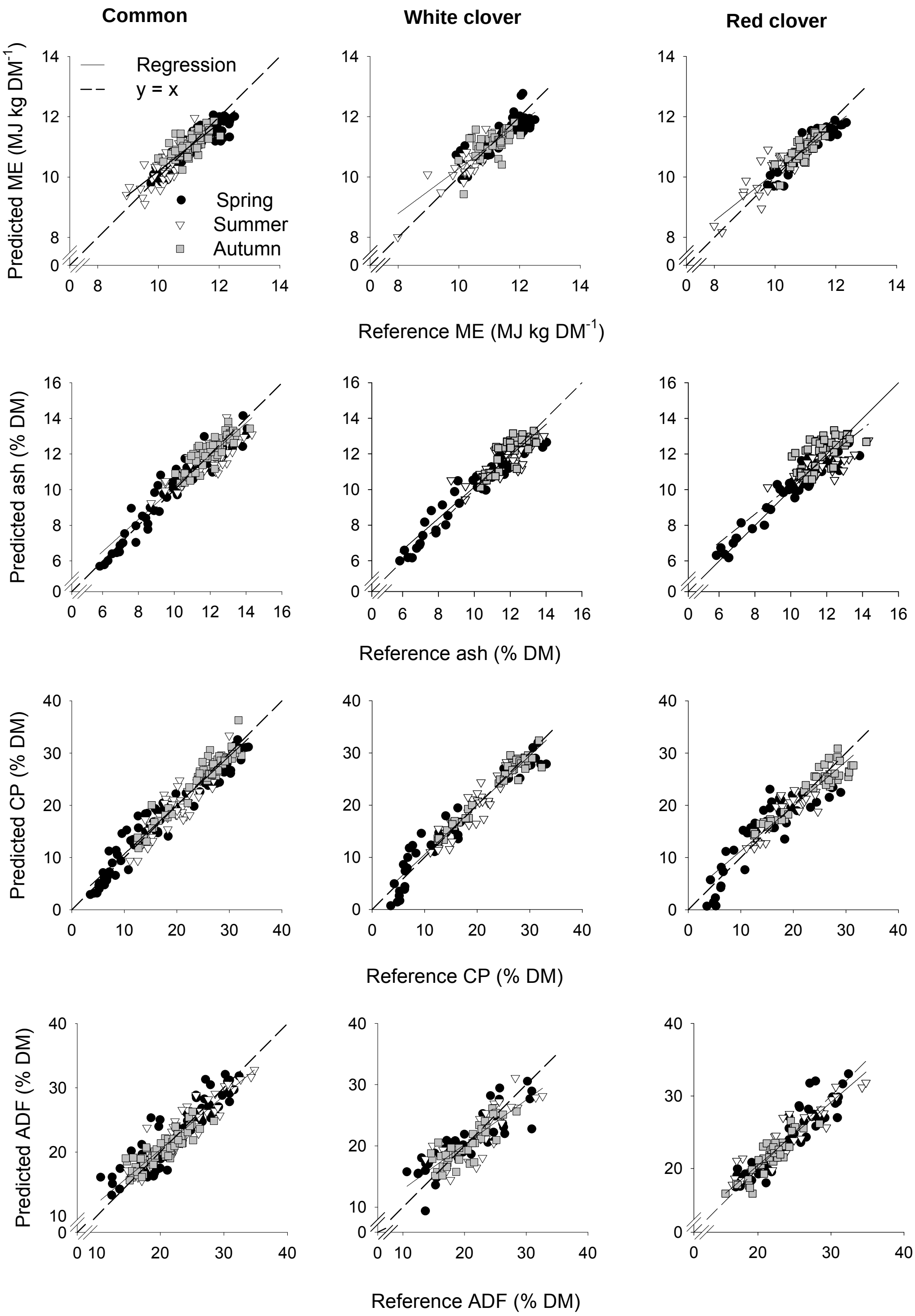

Figure 6. 2: Comparison of the reference values of metabolizable energy (ME), ash, crude protein (CP) and acid detergent fibre (ADF) as detected by laboratory NIRS and values predicted by field spectroscopy using modified partial least square regression (statistics see Table 6.4) for the common, white clover- and red clover-specific data set (legume-specific calibrations are composed by binary legume-grass mixtures, pure legume and pure grass swards).

rather low ADF values (13-15% DM). In the calibration procedure of ash only six outliers were eliminated, which were mainly in the advanced growth stages (BBCH 55-65). No specific pattern could be found for the four eliminated outliers in the CP model. The lower prediction accuracy of ME may be associated with its narrow range of values which was relatively higher for the other constituents. Overall, the better performance of MPLS compared with two-waveband ratios is in accordance with other authors (Cho et al., 2007; Hansen and Schjoerring, 2003), showing that MPLS indeed is a potentially useful method.

In practise legume-grass mixtures often exhibit a wide range of legume dry matter contribution which comprises all levels between pure legume and pure grass spots in parts of the field. Therefore, legume-specific calibrations were developed, which included mixtures and pure swards of perennial ryegrass and of the respective legume species. Legume-specific calibrations resulted in enhanced prediction accuracies in cross validation for ash and CP. Prediction accuracy for ME and ADF could not be improved (Figure 6. 2, Table 6. 4).

Prediction accuracy of SMLR resembled that of the MPLS models (Table 6. 5). In the calibration procedure slightly lower R^2 values were obtained, which were 0.73, 0.79, 0.87 and 0.75 for ME, ash, CP and ADF, respectively. However, in cross validation prediction accuracy was mostly higher, yielding in a SECV of 0.4, 0.8, 2.8 and 2.5 for ME, ash, CP and ADF, which is also reflected on the slightly higher RPD values.

Legume-specific calibrations resulted in an improved prediction accuracy for all nutritive variables except for ME. Furthermore, fewer outliers were detected in the SMLR than in the MPLS procedure (except with the ADF model). Wavebands selected were scattered over the whole spectrum and differed widely among the constituents and legume species which was also found in other investigations, where selected wavebands differed strongly among the plant species (Biewer et al., 2008; Huang et al., 2004, LaCapra et al., 1996; Thenkabail et al., 2000). There exists a multitude of possible explanations, including variability in the magnitudes of constituent levels between data sets, canopy architecture effects, sensor limitations, background vegetation and soil influences (Kokaly, 2001). Yet, Huang et al.

Table 6. 5: Calibration statistics for the prediction of metabolizable energy (ME), ash, crude protein (CP) and acid detergent fibre (ADF) by stepwise multiple linear regression and wavebands selected.

	Constituent	Selected wavebands	N	Calibration				Cross-validation		
				Mean	SD	SEC	R^2	SECV	1-VR	RPD
Common	ME, MJ kg^{-1} DM^{-1}	459, 555, <u>843</u>, 1359, 1471, 1675, <u>1739</u>, 2216	194	10.9	0.8	0.4	0.73	0.4	0.71	1.9
	Ash, % DM	371, 603, <u>695</u>, 1179, 1219, 1399, <u>1555</u>, 2084	197	11.3	1.8	0.8	0.79	0.8	0.78	2.2
	CP, % DM	399, <u>419</u>, 439, 799, 871, 1219, <u>1555</u>	196	20.6	7.5	2.7	0.87	2.8	0.86	2.7
	ADF,% DM	<u>551</u>, 583, 671, 771, 863, 1227, <u>1319</u>, 1503, 1587	195	22.0	4.9	2.5	0.75	2.5	0.73	1.9
Mixtures including pure legume and grass swards										
White clover	ME, MJ kg^{-1} DM^{-1}	<u>551</u>, 583, 671, 771, 863, 1227, <u>1319</u>, 1503, 1587	115	11.0	0.9	0.5	0.73	0.5	0.70	1.8
	Ash, % DM	<u>691</u>, 779, <u>1495</u>, 2172	117	11.1	2.0	0.8	0.84	0.8	0.83	2.4
	CP, % DM	415, 635, <u>711</u>, <u>919</u>, 1155, 1247, 1755, 2176	117	19.6	8.3	2.1	0.94	2.1	0.93	3.9
	ADF,% DM	359, <u>539</u>, 639, <u>803</u>, 811, 1203, 1503, 1727, 1767	115	20.8	4.8	2.1	0.81	2.2	0.79	2.2
Red clover	ME, MJ kg^{-1} DM^{-1}	<u>675</u>, <u>871</u>, 1475, 1611, 1675, 1679	115	10.7	0.7	0.4	0.70	0.4	0.68	1.8
	Ash, % DM	419, 527, <u>603</u>, <u>699</u>, 1415, 1555, 1751, 2104	117	11.0	1.8	0.7	0.84	0.8	0.83	2.4
	CP, % DM	783, 843, <u>1247</u>, <u>1747</u>, 2128, 2340	118	18.8	7.2	2.5	0.88	2.6	0.87	2.8
	ADF,% DM	391, 451, <u>791</u>, 859, 1007, <u>1331</u>, 1371	114	23.1	4.3	1.8	0.82	1.9	0.81	2.3

SEC: standard error of calibration; SECV: standard error of cross validation; 1- VR: coefficient of determination of cross-validation: RPD: ratio of standard deviation of the measured results to standard error of cross validation; Underlined numbers are indicating the two most important wavelengths for the prediction of quality variables.

(2004) concluded that SMLR appeared to give stable results. In our study, the red (620-750 nm) and short wave near infrared (750-1100 nm) wavebands were selected in each model, reaching mostly the highest F-values, which indicates their importance for model prediction. This is in accordance with other studies where reflectance of the red and near infrared was frequently found to have a close relationship with plant biochemical concentration (Nguyen et al., 2006; Gianelle and Guastella, 2007; Starks et al., 2006a).

6.3.4 Hyperspectral analysis of reduced spectral data

Due to the importance of red and short wave near infrared wavebands in detecting forage quality variables as indicated by SMLR, the hyperspectral data were reduced to a range of 620 to 1000 nm with a resolution of 10 nm. This range was chosen in accordance to the Yara N-sensor® (FS; Yara International ASA, Oslo, Norway) which is already used for site-specific fertilizer applications in practise.

In comparison to the full data set the reduction resulted in lower prediction accuracies for MPLS models, except for the common and red clover-specific model of CP (Table 6. 6). Ash and CP still obtained rather high coefficients of determination in the cross validation (1-VR) ranging from 0.55 to 0.86, whereas ME and ADF only reached values of $0.44 \leq 1\text{-VR} \leq 0.50$ and $0.44 \leq 1\text{-VR} \leq 0.56$, respectively. However, 10 from 12 models showed RPD values ≥1.4, indicating still satisfactory prediction accuracy.

Results of SMLR analysis resembled that of MPLS (Table 6. 6). Ash and CP were estimated with satisfactory and good results, whereas the prediction accuracy of ME and ADF was poor, indicating that further spectral regions should be used for model building. Legume-specific calibration only improved model accuracy of ash and CP leading to a RPD value higher than 1.8.

The analyses of the reduced data set by MPLS and SMLR indicated that it might be difficult to accurately predict forage ME and ADF. However, estimates of ash contents were satisfactory and the determination of forage CP produced comparable results to that of the full data set.

Table 6. 6: Cross validation statistics of the prediction for metabolizable energy (ME), ash, crude protein (CP) and acid detergent fibre (ADF) by modified partial least squares regression (MPLS) and stepwise multiple linear regression (SMLR) with the reduced data set (620 to 1000 nm, resolution 10 nm).

		MPLS			SMLR		
		SECV	1-VR	RPD	SECV	1-VR	RPD
Common	ME, MJ kg^{-1} DM^{-1}	0.5	0.50	1.4	0.5	0.51	1.4
	Ash, % DM	1.0	0.62	1.6	1.0	0.64	1.7
	CP, % DM	3.1	0.83	2.4	3.7	0.75	2.0
	ADF,% DM	3.4	0.50	1.4	3.4	0.54	1.5
Mixtures including pure legume and grass swards							
White clover	ME, MJ kg^{-1} DM^{-1}	0.6	0.49	1.4	0.6	0.51	1.4
	Ash, % DM	0.9	0.78	2.1	0.9	0.76	2.1
	CP, % DM	3.1	0.86	2.7	3.3	0.84	2.5
	ADF,% DM	3.7	0.44	1.3	3.4	0.54	1.5
Red clover	ME, MJ kg^{-1} DM^{-1}	0.5	0.44	1.3	0.6	0.41	1.3
	Ash, % DM	1.0	0.55	1.5	0.9	0.71	1.9
	CP, % DM	2.9	0.83	2.4	3.2	0.80	2.2
	ADF,% DM	2.9	0.56	1.5	2.6	0.60	1.6

SECV: standard error of cross validation; 1- VR: coefficient of determination of cross validation; RPD: ratio of standard deviation of the measured results to standard error of cross validation.

It should be pointed out that the reference data were determined by laboratory NIRS and, hence, incorporated prediction errors by themselves. Thus, accuracies reported in the present study indicate the lower boundaries of the spectral methodology and may be further improved using reference values determined chemically in the laboratory. Furthermore, the use of an additional sensor that measures the incoming radiation to adjust the reflectance signal to changing light conditions or the application of artificial light to obtain stable measurements which are free of weather interferences may still enhance prediction accuracy.

6.4 Conclusions

The developed two-waveband reflectance ratios, which were based on signals at specific wavelengths, had weak relationships with all forage quality constituents. Hyperspectral analysis by MPLS and SMLR resulted in the highest accuracy for the estimation of ME, ash, CP and ADF with standard errors of cross validation for the respective variables of 0.4, 0.9, 3.1 and 2.4 for MPLS and 0.4, 0.8, 2.8 and 2.5 for SMLR, respectively. Accuracy of prediction for ash, CP and to some extent for ADF was further improved by legume-specific calibrations. Although selected wavelengths by SMLR analysis differed for each calibration, in nearly all models the red and especially the near infrared region revealed the highest information for the prediction of nutritive variables. The analysis of the reduced hyperspectral data set to the range of 620 to 1000 nm with a resolution of 10 nm indicated that it might be difficult to accurately predict forage ME and ADF, whereas forage ash could be predicted with satisfactory and CP with good results.

7 General discussion

The objectives of this study were to evaluate if field spectral measurements can be used to predict the DM yield and the forage quality constituents ME, ash, CP and ADF of legume-grass swards across a wide range of legume proportion and growth stage. Two experiments were conducted in a greenhouse under controlled conditions which allowed to collect spectral measurements which were free from interferences such as wind, passing clouds and changing angles of solar irradiation. This initial investigation was then evaluated over two years in a field experiment with the same legume-grass swards in order to test its applicability for practical purposes.

7.1 General aspects of hyperspectral data analysis

Field spectroscopy has been widely investigated as analytical tool for the detection of nitrogen (Erasmi, 2002; Jain et al., 2007; Mutanga et al., 2003; Zhao et al., 2005), biomass (Hansen and Schjoerring, 2003; Starks et al., 2008; Zhao et al., 2003) and nutritive values (Starks et al., 2006a) of different agricultural crops. However, spectral signals of canopy reflectance are very complex as they are influenced by various factors such as biophysical and biochemical properties, sward architecture, canopy background (i.e. soil, litter), atmospheric conditions, incidence angle of light and sensor configuration. Hence, the challenge of spectral analysis is to minimize negative effects which interfere with the reflection signal as well as to identify those regions in a spectrum which are closely correlated to the vegetation parameter in question. However, the spectral determination of several parameters such as DM yield can rather be conducted by indirect attributes. Green biomass, for example, is closely correlated to the chlorophyll content of plants which causes considerable photosynthetic absorption of the incoming radiation in the visible red reflectance or to spongy mesophyll and plant cell structural material which is linked to the near infrared reflectance (Jensen, 2000). A difficulty in detecting biomass and other vegetation parameters is that chemical and structural compositions of plants change considerably during the growing period, entailing a change in their spectral reflectance. With plant maturation the fraction of dry biomass increases and the proportion of cell wall material augments in relation to cell contents. Loss of pigmentation i.e. enhances visible reflectance, particularly in the red region of the spectrum (Hoffer, 1978). Consequently, VIs which are based on the red and near infrared region are highly affected by sward maturation.

7.2 Options of hyperspectral data analysis

In our study hyperspectral data was analysed by the common known vegetation indices SR, NDVI, EVI and REP as well as by two waveband reflectance ratios using only a limited range of the whole spectrum. The two waveband reflectance ratios were developed according to Zhao et al. (2005) in order to find the best waveband ratio for the prediction of nutritive values. In addition MPLS and SMLR models were calculated including the whole hyperspectral data range.

The detection of DM yield by the vegetation indices SR, NDVI, EVI and REP were strongly interfered by sward maturation and the occurrence of larger areas of visible soil. Only in the first greenhouse experiment, where swards had large leaf to stem ratios, good prediction results could be obtained. For more mature and open swards VI-based detection of DM yield was not possible.

The development of two-waveband reflectance ratios, which were not necessarily based on the red and NIR but on signals at specific narrowbands, did also not succeed in predicting forage quality variables. The combination of heterogeneous sward structures caused by a wide range of developmental stages and a varying legume dry matter contribution, as well as variable atmospheric conditions while taking spectral measurements at field may have confounded the relationships between two-waveband reflectance ratios and quality constituents. Hence, the reduction of spectral information to only two spectral wavebands does not suffice to cover the high variability within the investigated swards.

In contrast, the use of several wavebands for the prediction of vegetation parameters by SMLR and MPLS solved the problem of changing spectral responses during the growing period. Especially with MPLS regression, including the whole spectrum into the analysis, good prediction results were obtained. Hence, the combination of many different absorption features of the spectrum may balance the effect of heterogeneous sward structures and variable atmospheric conditions.

Good results were also achieved with SMLR, although the hyperspectral data set was reduced to only a few wavebands. Nevertheless, uncertainty of the robustness of SMLR models is imposed by the different choice of wavebands in each model which is also found in other investigations in which selected wavebands differ strongly between the plant species (Huang et al., 2004; LaCapra et al., 1996; Thenkabail et al., 2000). Kokaly (2001) suggests that differences in the magnitudes of variable levels between data sets, canopy architecture effects, sensor limitations, background vegetation and soil influences are reasons for this finding. Furthermore, Martin and Aber (1997) and LaCapra et al. (1996) demonstrate that equations for estimating nitrogen derived from one site are unable to predict the nitrogen concentrations for other sites. Thus, to prove the robustness of SMLR models it is necessary to extend them to further data sets.

However, the aim of this study was to select some regions in the hyperspectral data range by SMLR that are closely correlated with DM yield and nutritive values of legume-grass swards. The red (620-750 nm) and short wave near infrared (750-1100 nm) wavebands were selected in each model, reaching mostly the highest F-values, which indicates their importance for model prediction. This choice was made in accordance with other studies where reflectance of the red and near infrared is frequently found to have a close relationship with DM yield (Nguyen et al., 2006; Thenkabail et al., 2000) and plant biochemical concentration (Gianelle and Guastella, 2007; Starks et al., 2006a). Hence, the hyperspectral data set was reduced to the range of the Yara N-sensor® which uses the red and short wave near infrared reflectance for site-specific fertiliser applications in practise. In comparison to the full data set the reduction resulted in lower prediction accuracy for almost all MPLS and SMLR models. Especially forage ME and ADF could be hardly predicted, showing that further spectral regions should be used for model building. However, estimates of ash content and DM yield were satisfactory and the determination of forage CP produced very good results which were comparable to that of the full data set.

Yet for the application of field spectral measurements in practise there is a balance to be found between reducing the spectral data range in order to lower the costs for the sensor and maintaining high prediction accuracy for DM yield and forage nutritive values. Sanderson et al. (2001) evaluate accurate assessment of forage mass in pastures with an electronic capacitance meter, a rising plate meter and a pasture ruler. They state that none of these indirect methods are accurate or precise and error levels range from 26 to 33% of the mean forage mass measured on the pastures. Different scenarios are then simulated, including under- or over-estimating forage yield on pastures by 10 or 20%. All scenarios simulated result in lower net returns compared with the optimum farm, with decreases in net return ranging from \$8 to \$198 $ha^{-1}yr^{-1}$. In our field study the relative error [(SECV of the model/mean of values included) 100] for the prediction of DM yield was even higher than 33% when the reduced data set was applied, indicating that model development must be improved.

Also the prediction accuracy of ash content should be enhanced, as the SECV (10.4 % DM) was as high as the difference between maximum and minimum ash value (10.1 % DM). However, the SECV of CP (3.1 % DM) was relatively small in comparison to the range of CP values (min. 6.0 and max. 33.6 % DM), indicating that CP can be determined quite well with the reduced data set. At the same time, the range of CP was very wide, as both very young swards with high CP contents and swards with considerably amounts of dry leaves, holding low CP contents, were investigated.

7.3 Options to improve the applicability of field spectral measurements

A possibility to improve the validity of spectral measurements was the calculation of legume-specific models, which included mixtures and pure swards of perennial ryegrass and of the respective legume species. Thereby differences in canopy structure, chemical composition and reflectance properties of plant species were considered. In the NIR region, for example, broad-leaved crops always result in larger reflectance values than small-leaved crops, whereas in the red region they show a similar trend in reflectance (Gao et al., 2000; Huete et al. 1997).

In a first attempt, the distinct reflectance properties of plant species were also used to determine the proportion of legumes in the swards using VIs. However, differences in reflectance characteristics only enabled the prediction of proportions of grass and legumes when the DM yield of the sward remained constant. With varying levels of DM yield, the relationship between spectral signature and species proportion in the swards was covered by the effect of DM yield on reflectance. The attempt to improve the determination of legume proportions by MPLS analysis also did not succeed. Since DM yield affected reflectance much more strongly than leaf shape and leaf orientation it must be concluded that, for a non-destructive assessment of legume proportion in mixed swards, more appropriate methods, e.g. linear spectral unmixing (Mewes et al. 2008) or digital image analysis (Himstedt et al., 2006) are necessary.

In this investigation the interaction between spectral reflectance and weather conditions as well as the incidence angle of light interfered the most with an accurate determination of DM yield. This can be clearly seen through the comparison of the greenhouse and the field experiments. In the greenhouse studies spectral recordings were taken under controlled conditions with artificial light sources, which resulted in considerably higher accuracy for DM yield prediction by MPLS, SMLR and VIs than in the field experiment. These results indicate the potentials for further improvements for the prediction of DM yield and forage quality constituents even under field conditions. This could for example be achieved by the use of an additional sensor that measures the incoming radiation to adjust the reflectance signal to changing light conditions or by the application of artificial light to obtain stable measurements which are free of weather interferences and incidence angle of light.

A further possibility to improve model calibration for the prediction of DM yield and forage quality constituents of legume-grass swards, could be the exclusion of reflectance data derived from swards that cut across the growth stage of early flowering. Spectral reflectance of swards at advanced growth stages interfered with all methods for hyperspectral data analysis. For practical purposes, however, they are of secondary significance as swards are commonly cut when grasses are at early head emergence and legumes are in the early stage of flowering (Frame, 1992).

It is also supposable that the occurrence of varying areas of visible soil impacted the accurate prediction of DM yield and forage quality constituents by spectral data, as it is also part of the reflection signal. However, these effects should not affect the application of field spectroscopy at the time of harvest, as by then the ground is usually completely covered by the plant canopy.

Beside these possibilities to improve prediction accuracy of field spectral measurements further research is still needed to evaluate the methods by effects of varying sites and vegetation periods in order to enhance the robustness and portability of models to other environmental conditions.

8 Conclusions

The following conclusions can be drawn from the two greenhouse experiments and the field study with pure stands and binary mixtures of different forage legumes and perennial and annual ryegrass:

i) The vegetation indices, SR, NDVI, EVI and REP, based on the red and near infrared wavelengths had weak relationships with DM yield. An exception is the first greenhouse experiment, where the swards did not reach the growth stage of flowering and DM yield was well predicted by all vegetation indices. In this case EVI proved to be the most appropriate index with the smallest standard errors and good accuracy even at higher biomass levels.

ii) The developed two-waveband reflectance ratios had weak relationships with the forage quality constituents ME, ash, CP and ADF.

iii) Hyperspectral analysis by MPLS and SMLR resulted for all experiments in the highest accuracy for the prediction of DM yield and the forage quality constituents ME, ash, CP and ADF.

iv) Although selected wavelengths by SMLR analysis differed for each calibration, in nearly all models the red and especially the near infrared region revealed the highest information for DM yield prediction and the determination of ME, ash, CP and ADF. The reduction of the hyperspectral data set to the range of 620 to 1000 nm with a resolution of 10 nm indicated that it is difficult to accurately predict forage ME and ADF, whereas DM yield, as well as forage ash could be predicted with satisfactory results and CP with good results.

v) Accuracy of prediction was further improved for all methods of spectral data analysis by legume-specific calibrations.

vi) The comparison between the greenhouse and the field experiments indicated that the interaction between spectral reflectance and weather conditions as well as incidence angle of light interfered with an accurate determination of DM yield. Hence, further improvements for the prediction of DM yield and forage quality constituents under field conditions should be tested. This may be achieved, for example, by the use of an additional sensor that measures the incoming radiation to adjust the reflectance signal to changing light conditions or by the application of artificial light to obtain stable measurements which are free of weather interferences.

The results of this study have shown the potential of field spectroscopy and proved its usefulness for predicting DM yield, ash content and CP across a wide range of legume species, legume proportion and growth stage. Further research is needed to evaluate the effects of changing weather conditions and incidence angle of light source on the reflection signal to develop strategies for the handling of these interferences. Furthermore, the developed models should be tested on varying sites and vegetation periods in order to enhance the robustness and portability of these models under other environmental conditions.

9 Summary

Productivity, botanical composition and forage quality of legume-grass swards are important factors for successful arable farming in both organic and conventional farming systems. As these attributes can vary considerably within a field, a non-destructive method of detection while doing other tasks would facilitate a more targeted management of crops, forage and nutrients in the soil-plant-animal system.

This study was undertaken to explore the potential of field spectral measurements for a non destructive prediction of dry matter (DM) yield, legume proportion in the sward, metabolizable energy (ME), ash content, crude protein (CP) and acid detergent fiber (ADF) of legume-grass mixtures. Two experiments were conducted in a greenhouse under controlled conditions which allowed to collect spectral measurements which were free from interferences such as wind, passing clouds and changing angles of solar irradiation. In a second step this initial investigation was evaluated in the field by a two year experiment with the same legume-grass swards. Several techniques for analysis of the hyperspectral data set (350-2500 nm) were examined in this study: four vegetation indices (VIs): simple ratio (SR), normalized difference vegetation index (NDVI), enhanced vegetation index (EVI) and red edge position (REP), two-waveband reflectance ratios, modified partial least squares (MPLS) regression and stepwise multiple linear regression (SMLR).

The first and second greenhouse experiments comprised a sample size of 80 and 192 experimental swards, respectively. Pure swards of red clover (*Trifolium pratense* L.), white clover (*Trifolium repens* L.) and lucerne (*Medicago sativa* L.) as well as binary mixtures of each legume with perennial ryegrass (*Lolium perenne* L.) in the first experiment and with annual ryegrass (*Lolium multiflorum* Lam. ssp. *alternativum*) in the second experiment were tested. Growth stages ranged from tillering to start of flowering and the proportion of legumes from 0 to 92 %. Dry matter yield prediction by MPLS and SMLR gave the largest R^2 values ranging from 0.85 to 0.99 with standard errors of cross validation (SECV) differing between 9 and 28 g DM m^{-2}. The prediction of DM yield by VIs resulted in R^2 values of 0.87 to 0.90 and standard errors of 4 to 20 g DM m^{-2} for swards with large leaf to stem ratios; EVI was the most accurate. For more mature and open swards VI-based detection of DM yield was not possible. The contribution of legumes to the sward could be determined at a constant DM yield level by the VIs, but this was not possible when the level of DM yield varied.

The two year field experiment represented a wide range of different legume-grass swards [(*Lolium perenne* (L.), *Trifolium pratense* (L.), *Trifolium repens* (L.), *Medicago sativa* (L.) and *Lotus corniculatus* (L.)], legume proportion (0 to 100% of DM) and growth stages (beginning of tillering to end of flowering). In total 459 legume-grass samples were investigated. Similar to the greenhouse experiments DM yield prediction by MPLS and SMLR gave the best R^2 values ranging in cross validation from 0.74 to 0.92 with a standard error below 92 g DM m^{-2}. The DM yield prediction by VIs resulted in unsatisfactory accuracies. Prediction accuracy for MPLS and SMLR models to determine DM yield in cross validation were still acceptable ($0.61 \leq R^2 \leq 0.88$; $70.0 \leq SECV \leq 114.2$) even with a reduced spectral data set (630 to 1000 nm with a resolution of 10 nm).

To explore the potential of field spectral measurements for the prediction of the forage quality constituents ME, ash, CP and ADF, the field data of the second year, comprising a population of 200 legume-grass swards [*Lolium perenne* (L.), *Trifolium repens* (L.), *Trifolium pratense* (L.)], was investigated. Forage quality constituents had weak relationships with the developed reflectance ratios, whereas hyperspectral analysis by MPLS and SMLR resulted in high prediction accuracy in cross validation ($0.70 \leq R^2 \leq 0.94$; $0.4 \leq SECV \leq 3.1$). Even with a reduced spectral data set (630 to 1000 nm) estimates of MPLS and SMLR models were still acceptable for forage ash ($0.62 \leq R^2 \leq 0.78$; $0.9 \leq SECV \leq 1.0$) and CP ($0.83 \leq R^2 \leq 0.86$; $2.9 \leq SECV \leq 3.7$) in cross validation.

Field spectroscopy has shown its potential and proved its usefulness for the prediction of DM yield, ash content and CP across a wide range of legume proportion and growth stage. In all investigations prediction accuracy of DM yield, ash content and CP could be improved by legume-specific calibrations which included mixtures and pure swards of perennial ryegrass and of the respective legume species. The comparison between the greenhouse and the field experiments shows that the interaction between spectral reflectance and weather conditions as well as incidence angle of light interfered with an accurate determination of DM yield. Further research is hence needed to improve the validity of spectral measurements in the field. Furthermore, the developed models should be tested on varying sites and vegetation periods to enhance the robustness and portability of the models to other environmental conditions.

10 Zusammenfassung

Sowohl in der ökologischen als auch in der konventionellen Landwirtschaft sind Produktivität, Bestandszusammensetzung und Futterqualität von Leguminosengras-Beständen wichtige Voraussetzungen für einen erfolgreichen Feldfutterbau. Diese Parameter können jedoch innerhalb eines Feldes beachtlichen Schwankungen unterworfen sein, so dass eine nicht destruktive Erfassung von Produktivität, Bestandszusammensetzung und Futterqualität während der Feldarbeit ein verbessertes Management der Leguminosengras-Bestände, der Fütterung sowie der Düngung ermöglichen würde.

Die vorliegende Untersuchung evaluiert das Potenzial feldspektroskopischer Messungen für die Erfassung des Trockenmasseertrags (TM-Ertrags), des Leguminosenanteils in den Pflanzenbeständen, der metabolisierbaren Energie (ME), des Aschegehalts, des Rohproteins (RP) und des Acid Detergent Fibre (ADF) von Leguminosengras-Beständen. Dafür wurden zwei Gewächshausversuche durchgeführt, die es ermöglichten, die spektroskopischen Messungen unter kontrollierten Bedingungen durchzuführen: frei von Störungen durch Wind, vorbeiziehende Wolken und sich ändernde Winkel der Sonneneinstrahlung. Des Weiteren wurde ein zweijähriger Freilandversuch mit den gleichen Leguminosengras-Beständen durchgeführt, um den Einsatz feldspektroskopischer Messungen unter Praxisbedingungen zu prüfen. Verschiedene Methoden zur Auswertung der hyperspektralen Datensätze wurden untersucht: vier Vegetationsindizes: Simple Ratio (SR), Normalized Difference Vegetation Index (NDVI), Enhanced Vegetation Index (EVI) und Red Edge Position (REP), die Entwicklung von Reflexionsquotienten aus den Messwerten zweier Wellenlängenbereiche, Modified Partial Least Squares (MPLS) Regression und Stepwise Multiple Linear Regression (SMLR).

Der erste und zweite Gewächshausversuch umfaßte einen Probenumfang von 80 bzw. 192 Leguminosengras-Beständen. Untersucht wurden Reinsaaten und binäre Leguminosengras-Gemenge aus Rotklee (*Trifolium pratense* L.), Weißklee (*Trifolium repens* L.), Luzerne (*Medicago sativa* L.) und Deutschem Weidelgras (*Loli-*

um perenne L.) im ersten Versuch, sowie Einjährigem Weidelgras (*Lolium multiflorum* Lam. ssp. *alternativum*) im zweiten Versuch. Die untersuchten Bestände umfassten Vegetationsstadien von der Bestockung/Bildung von Seitensprossen bis zur Blüte. Der Anteil der Leguminosen in den Pflanzenbeständen schwankte zwischen 0 und 92%. Mit MPLS und SMLR konnte der TM-Ertrag am Besten bestimmt werden, die Werte des Bestimmtheitsmaßes (R^2) lagen dabei zwischen 0.85 und 0.99 und der Standardfehler der Kreuzvalidation (SECV) zwischen 9 und 28 g TM m^{-2}. Die Schätzung des TM-Ertrags durch die VIs ergab im ersten Gewächshausversuch mit Pflanzenbeständen, die ein hohes Blatt-zu-Stängel-Verhältnis aufwiesen, gute Ergebnisse ($0.87 \leq R^2 \leq 0.9$ und $4 \leq$Standardfehler ≤ 20), wobei die größte Genauigkeit mit dem EVI erzielt wurde. Dagegen war die TM-Ertragsschätzung durch VIs im zweiten Gewächshausversuch bei älteren und gleichzeitig lichteren Pflanzenbeständen nicht möglich. Der Leguminosenanteil im Pflanzenbestand konnte nur bei einem konstanten TM-Ertrag bestimmt werden.

Im zweijährigen Feldversuch wurden Reinsaaten und binäre Gemenge aus *Lolium perenne* (L.), *Trifolium pratense* (L.), *Trifolium repens* (L.), *Medicago sativa* (L.) und *Lotus corniculatus* (L.) untersucht. Insgesamt wurden 459 Leguminosengras-Proben mit unterschiedlichen Leguminosenanteilen in den Pflanzenbeständen (0-100%) und Vegetationsstadien (Anfang Bestockung/Bildung von Seitensprossen bis Ende Blüte) erhoben. Ähnlich wie bei den Gewächshausversuchen, wurden mit MPLS und SMLR die besten Ergebnisse erzielt ($0.74 \leq R^2 \leq 0.92$; SECV<92 g TM m^{-2}). Mit den VIs konnte der TM-Ertrag nur unbefriedigend bestimmt werden. Trotz der TM-Ertragsbestimmung durch einen reduzierten Spektraldatensatz (630 bis 1000 nm mit einer Auflösung von 10 nm) waren die Ergebnisse der Kreuzvalidation für MPLS und SMLR befriedigend ($0.61 \leq R^2 \leq 0.88$; $70.0 \leq$SECV≤ 114.2).

Um das Potenzial feldspektroskopischer Messungen für die Erfassung der Futterqualitätsparameter ME, Aschegehalt, RP und ADF zu bestimmen, wurden die Felddaten aus dem zweiten Freiland-Versuchsjahr untersucht. Dafür wurden insgesamt 200 Proben von Reinsaaten und binären Gemengen aus *Lolium perenne* (L.), *Trifolium repens* (L.) und *Trifolium pratense* (L.) verwendet. Die Futterqualitätsparameter und die entsprechend entwickelten Reflexionsquotienten waren nur schwach miteinander korreliert. Dagegen führte die hyperspektrale Datenauswertung mittels MPLS und SMLR zu guten Ergebnissen in der Kreuzvalidation ($0.70 \leq R^2 \leq 0.94$; $0.4 \leq SECV \leq 3.1$). Die Schätzergebnisse der MPLS und SMLR in der Kreuzvalidation waren mit dem reduzierten Spektraldatensatz (630 bis 1000 nm mit einer Auflösung von 10 nm) für den Aschegehalt ($0.62 \leq R^2 \leq 0.78$; $0.9 \leq SECV \leq 1.0$) und für RP ($0.83 \leq R^2 \leq 0.86$; $2.9 \leq SECV \leq 3.7$) befriedigend bzw. gut.

Die vorliegende Untersuchung bestätigt die Eignung feldspektroskopischer Messungen für die Bestimmung des TM-Ertrags, des Aschegehalts und des Rohproteins. Die Schätzgenauigkeit dieser Parameter konnte in allen Untersuchungen durch leguminosen-spezifische Kalibrationen verbessert werden. Der Vergleich zwischen Gewächshaus- und Freilandversuch zeigt, dass Änderungen der Witterung sowie des Sonneneinstrahlungswinkels die genaue Bestimmung des TM-Ertrags erschweren. Weitere Untersuchungen sind nötig, um die Güte spektraler Messungen im Feld zu verbessern. Darüber hinaus wäre es sinnvoll, die Robustheit und Übertragbarkeit der Modelle auf andere Umweltbedingungen zu prüfen und gegebenenfalls in unterschiedlichen Gegenden und Vegetationsperioden weiter zu entwickeln.

11 References

Bailey, J.S., K. Wang, C. Jordan and A. Higgins. 2001. Use of precision agriculture technology to investigate spatial variability in nitrogen yields in cut grassland. Chemosphere 42: 131-140.

Berardo, N. 1997. Prediction of the chemical composition of white clover by near-infrared reflectance spectroscopy. Grass and Forage Science 52: 27-32.

Biewer, S., S. Erasmi, T. Fricke and M. Wachendorf. 2008. Prediction of yield and the contribution of legumes in legume-grass mixtures using field spectrometry. Precision Agriculture, Online first. DOI 10.1007/s11119-008-9078-9.

Blackburn, A. 1998. Quantifying chlorophylls and caroteniods at leaf and canopy scales: an evaluation of some hyperspectral approaches. Remote Sensing of Environment 66: 273-285.

Blackburn, G.A. and J.I. Pitman. 1999. Biophysical controls on the directional spectral reflectance properties of bracken (*Pteridium aquilinum*) canopies: results of a field experiment. International Journal of Remote Sensing 20 (11): 2265-2282.

Blackburn, G.A. and C.M. Steele. 1999. Towards the remote sensing of matorral vegetation physiology: relationships between spectral reflectance, pigment, and biophysical characteristics of semiarid bushland canopies. Remote Sensing of Environment 70: 278-292.

Boegh, E., H. Soegaard, N. Broge, C.B. Hasager, N.O. Jensen, K. Schelde and A. Thomsen. 2002. Airborne multispectral data for quantifying leaf area index, nitrogen concentration, and photosynthetic efficiency in agriculture. Remote Sensing of Environment 81: 179-193.

Boller, B.C. and J. Nösberger. 1987. Symbiotically fixed nitrogen from field-grown white and red clover mixed with ryegrasses at low levels of ^{15}N-fertilization. Plant and Soil 104:219-226.

Boschetti, M., S. Bocchi and P.A. Brivio. 2007. Assessment of pasture production in the Italian Alps using spectrometric and remote sensing. Agriculture, Ecosystems and Environment 118: 267-272.

Box E.O., B.N. Holben and V. Kalb. 1989. Accuracy of the AVHRR vegetation index as a predictor of biomass, primary productivity and net CO_2 flux. Plant Ecology 80: 71-89.

Carter, G.A. and B.A. Spiering. 2002. Optical properties of intact leaves for estimating chlorophyll concentration. Journal of Environmental Quality 31: 1424-1432.

Cho, M.A. 2007. Hyperspectral remote sensing of biochemical and biophysical parameters: The derivative red-edge double-peak feature, a nuisance or an opportunity? PhD. thesis Wageningen University, the Netherlands.

Cho, M.A., A. Skidmore, F. Corsi, S.E. van Wieren and I. Sobhan. 2007. Estimation of green/herb biomass from airborne hyperspectral imagery using spectral indices and partial least squares regression. International Journal of Applied Earth Observation and Geoinformation 9: 414-424.

Clark, R.N. 1999. Spectroscopy of rocks and minerals and principles of spectroscopy. In: Rencz, A. N. (ed.): Remote Sensing for the Earth Sciences: Manual of Remote Sensing (pp. 3-58). 3. ed., Wiley, New York, USA.

Clevers, J.G.P.W., S.M. de Jong, G.F. Epema, F. Van der Meer, W.H. Bakker, A.K. Skidmore and E.A. Addink. 2001. MERIS and the red-edge position. International Journal of Applied Earth Observation and Geoinformation, 3 (4): 313-319.

Cozzolino, D., A. Fassio, E. Fernandez, E. Restaino and A. La Manna. 2006. Measurement of chemical composition in wet whole maize silage by visible and near infrared reflectance spectroscopy. Animal Feed Science and Technology 129: 329-336.

Curran, P.J. 1983. Estimating green LAI from multispectral aerial photography. Photogrammetric Engineering and Remote Sensing 49: 1709-1720.

Curran, P.J., J.L. Dungan, B.A. Macler, S.E. Plummer and D.L. Peterson. 1992. Reflectance spectroscopy of fresh whole leaves for the estimation of chemical concentration. Remote Sensing of Environment 39: 153-166.

Dawson, T.P. and P.J. Curran. 1998. A new technique for interpolating the reflectance red edge position. International Journal of Remote Sensing, 19 (11): 2133-2139.

De Boever, J.L., B.G. Cottyn, D.L. De Brabander, J.M. Vanacker and C.V. Boucqué. 1996. Prediction of the feeding value of grass silages by chemical parameters, in vitro digestibility and near-infrared reflectance spectroscopy. Animal Feed Science Technology 60: 103-115.

Dymond, J.R., J.D. Shepherd, H. Clark and A. Litherland. 2006. Use of vegetation satellite imagery to map pasture quality for input to a methane budget of New Zealand. International Journal of Remote Sensing 27 (6): 1261-1268.

Eastwood, J.A., M.G. Yates, A.G. Thomson and R.M. Fuller. 1997. The reliability of vegetation indices for monitoring saltmarsh vegetation cover. International Journal of Remote Sensing, 18 (18): 3901-3907.

Ehlert, D., V.C. Hammen and R. Adamek. 2003. On-line sensor pendulum meter for determination of plant mass. Precision Agriculture 4: 139-148.

Ehsani, M.R., S.K. Upadhyaya and D. Slaughter. 1999. A NIR technique for rapid determination of soil mineral nitrogen. Precision Agriculture 1: 217-234.

Elvidge, C.D. and Z. Chen. 1995. Comparison of broad-band and narrow-band red and near-infrared vegetation indices. Remote Sensing of Environment 54: 38-48.

Erasmi, S.and E.S. Dobers. 2004. Potential and limitations of spectral reflectance measurements for the estimation of the site specific variability in crops. p. 42-51). *In*: M. Owe and G. D'Urso (Eds.) Remote Sensing for Agriculture, Ecosystems and Hydrology V. Proc. of SPIE Vol. 5232. Barcelona, Spain. 8. Sept. 2003. Bellingham, WA USA.

Erasmi, S. 2002. Analyse spektroradiometrischer *in situ* Messungen als Datenquelle für die teilflächenspezifische Zustandsbeschreibung von Winterweizenbeständen. PhD. thesis Univ. of Göttingen, Germany.

Fassio, A. and D. Cozzolino. 2004. Non-destructive prediction of chemical composition in sunflower seeds by near infrared spectroscopy. Industrial Crops and Products 20: 321-329.

Ferreira, L.G., H. Yoshioka, A. Huete and E.E. Sano. 2003. Seasonal landscape and spectral vegetation index dynamics in the Brazilian Cerrado: An analysis within the large-scale biosphere-atmosphere experiment in Amazonia (LBA). Remote Sensing of Environment 87: 534-550.

Fitzgerald, G.J., D. Rodriguez, L.K. Christensen, R. Belford, V.O. Sadras and T.R. Clarke. 2006. Spectral and thermal sensing for nitrogen and water status in rainfed and irrigated wheat environments. Precision Agriculture 7: 233-248.

Frame, J. 1992. Improved grassland management. Farming Press Books, Ipswich, United Kingdom.

Friedl, M.A., J. Michaelsen, F.W. Davis, H. Walker and D.S. Schimel. 1994. Estimating grassland biomass and leaf area index using ground and satellite data. International Journal of Remote Sensing 15: 1401-1420.

Gamon, J.A., C.B. Field, M.L. Goulden, K.L. Griffin, A.E. Hartley, G. Joel, J. Peñuelas and R. Valentini. 1995. Relationships between NDVI, canopy structure, and photosynthesis in three Californian vegetation types. Ecological Applications 5: 28-41.

Gao, X., A.R. Huete, W. Ni and T. Miura. 2000. Optical-biophysical relationships of vegetation spectra without background contamination. Remote Sensing of Environment 74: 609-620.

Gausmann, H.W., W.A. Allen and R. Cardenas. 1969. Reflectance of cotton leaves and their structure. Remote Sensing of Environment 1: 19-22.

Geladi, P. and B.R. Kowalski. 1986. Partial least-squares regression: a tutorial. Analytica Chimica Acta 185: 1-17.

Gianelle, D. and F. Guastella. 2007. Nadir and off-nadir hyperspectral field data: strengths and limitations in estimating grassland biophysical characteristics. International Journal of Remote Sensing 28: 1547-1560.

Gottardi, S. 2008. Flächenhafte und funktionale Analyse kleinräumiger standortbedingter Variationen der Grünlandvegetation und -produktivität. PhD. thesis Univ. of Kassel, Germany.

Grossman, Y.L., S.L. Ustin, S. Jacquemoud, E.W. Sanderson, G. Schmuck and J. Verdebout. 1996. Critique of stepwise multiple linear regression for the extraction of leaf biochemistry information from leaf reflectance data. Remote Sensing of Environment 56: 182-193.

Günzler, H. and H.-U. Gremlich. 2003. IR-Spektroskopie. Eine Einführung. Wiley-VCH, Weinheim, Germany.

Haaland, D.M.a nd E.V. Thomas. 1988. Partial least-squares methods for spectral analyses. 2. Application to simulated and glass spectral data. Analytical Chemistry 60: 1202-1208.

Hansen, P.M. and J.K. Schjoerring. 2003. Reflectance measurement of canopy biomass and nitrogen status in wheat crops using normalized difference vegetation indices and partial least squares regression. Remote Sensing of Environment 86: 542-553.

Heege, H.J., S. Reusch and E. Thiessen. 2008. Prospects and results for optical systems for site-specific on-the-go control of nitrogen-top-dressing in Germany. Precision Agriculture 9: 115-131.

Heichel, G.H. and K.I. Henjum. 1991. Dinitrogen fixation, nitrogen transfer, and productivity of forage legume-grass communities. Crop Science 31: 202-208.

Hildebrandt, G. 1996. Fernerkundung und Luftbildmessung: für Forstwirtschaft, Vegetationskartierung und Landschaftsökologie. Wichmann, Heidelberg, Germany.

Himstedt, M., T. Fricke and M. Wachendorf. 2006. Image analysis as a tool to evaluate legume proportions in legume-grass mixtures. In A. Hellgadottir and E.M. Pötsch (Eds.), Quality Legume-Based Forage Systems for Contrasting Environments, Gumpenstein Proceedings COST 852 (pp. 165–168). Wallig-Ennstaler Druckerei und Verlag GmbH, Austria.

Hoffer, R.M. 1978. Biological and physical considerations in applying computer-aided analysis techniques to remote sensor data. In P. H. Swainand S. M. Davis (Eds.), Remote sensing: The quantitative approach (pp. 227–289). McGraw-Hill, New York, USA.

Høgh-Jensen, H., R. Loges, F.V. Jorgensen, F.P. Vinther and E.S. Jensen. 2004. An empirical model for quantification of symbiotic nitrogen fixation in grass-clover mixtures. Agricultural Systems 82: 181-194.

Horler, D.N.H., M. Dockray and J. Barber. 1983. The red edge of plant leaf reflectance. International Journal of Remote Sensing 4(2): 273-288.

Huang, Z., B.J. Turner, S.J. Dury, I.R. Wallis and W.J. Foley. 2004. Estimating foliage nitrogen concentration from HYMAP data using continuum removal analysis. Remote Sensing of Environment 93: 18-29.

Huete, A.R. 1988. A soil-adjusted vegetation index (SAVI). Remote Sensing of Environment 25: 295-309.

Huete, A.R. and R.D. Jackson. 1988. Soil and atmosphere influences on the spectra of partial canopies. Remote Sensing of Environment 25 (1): 89-105.

Huete, A.R., H.Q. Liu, K. Batchily and W. van Leeuwen. 1997. A comparison of vegetation indices over a global set of TM images for EOS-MODIS. Remote Sensing of Environment 59: 440-451.

Huete, A., K. Didan, T. Miura, E.P. Rodriguez, X. Gao and L.G. Ferreira. 2002. Overview of the radiometric and biophysical performance of the MODIS vegetation indices. Remote Sensing of Environment 83: 195-213.

Huete, A.R., K. Didan, Y.E. Shimabukuro, P. Ratana, S.R. Saleska, L.R. Hutyra, W. Yang, R.R. Nemani and R. Myneni. 2006. Amazon rainforests green-up with sunlight in dry season. Geophysical Research Letters 33: 1-4.

Ikeda, H., K. Okamoto and M. Fukuhara. 1999. Estimation of aboveground grassland phytomass with a growth model using Landsat TM and climate data. International Journal of Remote Sensing 20: 2283-2294.

Jain, N., S.S. Ray, J.P. Singh and S. Panigrahy. 2007. Use of hyperspectral data to assess the effects of different nitrogen applications on a potato crop. Precision Agriculture 8: 225-239.

Jensen, J.R. 2000. Remote sensing of the environment: An earth resource perspective. Prentice Hall, Upper Saddle River, New Jersey, USA.

Kokaly, R.F. and R.N. Clark. 1999. Spectroscopic determination of leaf biochemistry using band-depth analysis of absorption features and stepwise multiple linear regression. Remote Sensing of Environment 67: 267-287.

Kokaly, R.F. 2001. Investigating a physical basis for spectroscopic estimates of leaf nitrogen concentration. Remote Sensing of Environment 75: 153-161.

Künnemeyer, R., P.N. Schaare and M.M. Hanna. 2001. A simple reflectometer for on-farm pasture assessment. Computers and Electronics in Agriculture 31: 125-136.

Kumhála, F., M. Kroulík, J. Mašek and V. Prošek. 2003. Development and testing of two methods for the measurement of the mowing machine feed rate. Plant Soil Environment 49: 519-524.

Kumhála, F. and V. Prosek. 2003. Laboratory measurement of mowing machine material feed rate. Precision Agriculture 4: 413-419.

LaCapra, V.C., J.M. Melack, M. Gastil and D. Valeriano. 1996. Remote sensing of foliar chemistry of inundated rice with imaging spectrometry. Remote Sensing of Environment 55: 50-58.

Lamb, D.W., M. Steyn-Ross, P. Schaare, M.M. Hanna, W. Silvester and A. Steyn-Ross. 2002. Estimating leaf nitrogen concentration in ryegrass (*Lolium* spp.) pasture using the chlorophyll red-edge: theoretical modelling and experimental observations. International Journal of Remote Sensing 18: 3619-3648.

Lantinga, E.A., M. Nassiri and M.J. Kropff. 1999. Modelling and measuring vertical light absorption within grassclover mixtures. Agricultural and Forest Meteorology 96: 71-83.

Leardi, R. and A.L. Gonzalez. 1998. Genetic algorithms applied to feature selection in PLS regression: how and when to use them. Chemometrics and Intelligent Laboratory System 41: 195-207.

Ledgard, S. F. and K.W. Steele. 1992. Biological nitrogen fixation in mixed legume/grass pastures. Plant and Soil 141: 137-153.

Locher, F. 2003. Near infrared reflectance spectroscopy to predict the legume content in legume-grass mixture as a key parameter in N_2-fixation - method development, validation and application. PhD. thesis Univ. of München; Shaker Verlag GmbH, Aachen, Germany.

Loges, R. 1998. Ertrag, Futterqualität, N_2-Fixierungsleistung und Vorfruchtwert von Rotklee- und Rotkleegrasbeständen. PhD. thesis Univ. of Kiel; Schriftenreihe des Instituts für Pflanzenbau und Pflanzenzüchtung der Christian-Albrecht-Universität zu Kiel, Germany.

Martin, M.E. and J.D. Aber.1997. High spectral resolution remote sensing of forest canopy lignin, nitrogen, and ecosystem processes. Ecological Applications 7: 431-443.

Meier, U. 2001. Growth stages of mono- and dicotyledonous plants. BBCH monograph (2. ed.). Federal biological research centre for agriculture and forestry, Braunschweig, Germany.

Mewes, T., S. Erasmi, M. Kappas, S. Biewer, T. Fricke and M. Wachendorf. 2008. Spektrale Entmischung von Reflexionssignaturen zur Ermittlung von Leguminosenanteilen in Futterbaubeständen. Photogrammetrie, Fernerkundung, Geoinformation 4: 265-275.

Middleton, E.M., 1991. Solar zenith angle effects on vegetation indices in tallgrass prairie. Remote Sensing of Environment, 38 (1): 45-62.

Miller, C.E. 2001. Chemical principles of near-infrared technology. In: Williams, P. and K. Norris (Eds.). Near-infrared technology in the agricultural and food industries (2. ed. pp. 19-37). American Association of Cereal Chemists, Minesota, USA.

Montes, J.M., H.F. Utz, W. Schipprack, B. Kusterer, J. Muminovic, C. Paul and A.E. Melchinger. 2006. Near-infrared spectroscopy on combine harvesters to measure maize grain dry matter content and quality parameters. Plant Breeding 125: 591-595.

Mutanga, O., A.K. Skidmore and S. van Wieren. 2003. Discriminating tropical grass (*Cenchrus ciliaris*) canopies grown under different nitrogen treatments using spectroradiometry. Journal of Photogrammetry and Remote Sensing 57: 263-272.

Nguyen, H.T., J.H. Kim, A.T. Nguyen, L.T. Nguyen, J.C. Shin and B.-W. Lee. 2006. Using canopy reflectance and partial least squares regression to calculate within-field statistical variation in crop growth and nitrogen status of rice. Precision Agriculture 7: 249-264.

Nie, Z., J. Han, T. Liu and X. Liu. 2008. Hot topic: application of support vector machine method in prediction of alfalfa protein fractions by near infrared reflectance spectroscopy. Journal of Dairy Science 91 (6): 2361-2369.

Norris, K.H., R.F. Barnes, J.E. Moore and J.S. Shenk. 1976. Predicting forage quality by infrared reflectance spectroscopy. Journal of Animal Science 43: 889-897.

Numata, I., D.A. Roberts, O.A. Chadwick, J. Schimel, F.R. Sampaio, F.C. Leonidas and J.V. Soares. 2007. Characterization of pasture biophysical properties and the impact of grazing intensity using remotely sensed data. Remote Sensing of Environment 109: 314-327.

Park, R.S., F.J. Gordon, R.E. Agnew, R.J. Barnes and R.W.J. Steen. 1997. The use of near infrared reflectance spectroscopy on dried samples to predict biological parameters of grass silage. Animal Feed Science Technology 68: 235-246.

Pinar, A. and P. J. Curran. 1996. Technical Note: Grass chlorophyll and the reflectance red edge. International Journal of Remote Sensing 17 (2): 351-357.

Pu, R., P. Gong, G.S. Biging and M.R. Larrieu. 2003. Extraction of red edge optical parameters from hyperion data for estimation of forest leaf area index. IEEE Transactions on Geoscience and Remote Sensing 41: 916-921.

Qi, J., M.S. Moran, F. Cabot and G. Dedieu. 1995. Normalization of sun/view angle effects using spectral albedo-based vegetation indices. Remote Sensing of Environment 52 (3): 207-217.

Ray, S.S., G. Das, J.P. Singh and S. Panigrahy. 2006. Evaluation of hyperspectral indices for LAI estimation and discrimination of potato crop under different irrigation treatments. International Journal of Remote Sensing 27: 5373-5387.

Rudzik, L. 1993. Infrarotfibel. Infrarotspektroskopie für die Lebensmittel- und Milchindustrie. B. Behrs Verlag, Hamburg, Germany.

Sanderson, M.A., C.A. Rotz, S.W. Fultz and E.B. Rayburn. 2001. Estimating forage mass with a commercial capacitance meter, rising plate meter, and pasture ruler. Agronomy Journal 93: 1281-1286.

Sanderson, M.A., K.J. Soder, N. Brzezinski, F. Taube, K. Klement, L.D. Muller and M. Wachendorf. 2006. Sward structure of simple and complex mixtures of temperate forages. Agronomy Journal 98: 238-244.

SAS Institute. 2002-2003. SAS version 9.1. SAS Institute. Cary, NC, USA.

Savitzky, A. and M.J.E. Golay. 1964. Smoothing and differentiation of data by simplified least squares procedures. Analytical Chemistry 36: 1627-1639.

Schellberg, J., M.J. Hill, R. Gerhards, M. Rothmund and M. Braun. 2008. Precision agriculture on grassland: applications, perspectives and constraints. European Journal of Agronomy 29: 59–71.

Schilling, G. 2000. Pflanzenernährung und Düngung. Ulmer, Stuttgart.

Schino, G., F. Borfecchia, L. De Cecco, C. Dibari, M. Iannetta, S. Martini and F. Pedrotti. 2003. Satellite estimate of grass biomass in a mountainous range in central Italy. Agroforestry Systems 59: 157-162.

Schmidtke, K. 1997. Einfluss von Rotklee (*Trifolium pratense* L.) in Reinsaat und Gemenge mit Poaceen auf symbiontische N_2-Fixierung, bodenbürtige N-Aufnahme und $CaCl_2$-extrahierbare N-Fraktionen im Boden. PhD. thesis Univ. of Gießen; Eigenverlag Schmidtke, Germany.

Schmidtlein, S. nd J. Sassin. 2004. Mapping of continuous floristic gradients in grasslands using hyperspectral imagery. Remote Sensing of Environment 92: 126-138.

Schwinning, S. and A.J. Parsons. 1996. A spatially explicit population model of stoloniferous N-fixing legumes in mixed pasture with grass. Journal of Ecology 84: 815-826.

Sellers, P.J. 1985. Canopy reflectance, photosynthesis and transpiration. International Journal of Remote Sensing 6 (8): 1335-1372.

Serrano, L., J. Peuelas and S.L. Ustin. 2002. Remote sensing of nitrogen and lignin in Mediterranean vegetation from AVIRIS data: Decomposing biochemical from structural signals. Remote Sensing of Environment 81: 355-364.

Shenk, J.S. and M.O. Westerhaus.1993. Near infrared reflectance analysis with single and multiproduct calibrations. Crop Science 33: 582-584.

Sleugh, B., K.J. Moore, J.R. George and E.C. Brummer. 2000. Binary legume-grass mixtures improve forage yield, quality, and seasonal distribution. Agronomy Journal 92: 24-29.

Starks, P.J., S.W. Coleman and W.A. Phillips. 2004. Determination of forage chemical composition using remote sensing. Journal of Range Management 57: 635-640.

Starks, P.J., D. Zhao, W.A. Phillips and S.W. Coleman. 2006a. Development of canopy reflectance algorithms for real-time prediction of bermudagrass pasture biomass and nutritive values. Crop Science 46: 927-934.

Starks, P.J., D. Zhao, W. A. Phillips and S.W. Coleman. 2006b. Herbage mass, nutritive value and canopy spectral reflectance of bermudagrass pastures. Grass and Forage Science 61: 101-111.

Starks, P.J., D. Zhao and M.A. Brown. 2008. Estimation of nitrogen concentration and in vitro dry matter digestibility of herbage of warm-season grass pastures from canopy hyperspectral reflectance measurements. Grass and Forage Science 63: 168-178.

Terhoeven-Urselmans, T., K. Michel, M. Helfrich, H. Flessa and B. Ludwig. 2006. Near-infrared spectroscopy can predict the composition of organic matter in soil and litter. Journal of Plant Nutrition and Soil Science 169: 168-174.

Thenkabail, P.S., R.B. Smith and E. De Pauw, E. 2000. Hyperspectral vegetation indices and their relationships with agricultural crop characteristics. Remote Sensing of Environment 71: 158-182.

Thenkabail, P.S., E.A. Enclona, M.S. Ashton and B. van Der Meer. 2004. Accuracy assessments of hyperspectral waveband performance for vegetation analysis applications. Remote Sensing of Environment 91: 354-376.

Tillmann, P. 2000. Kalibrationsentwicklung für NIRS-Geräte – Eine Einführung. Cuvillier Verlag, Göttingen.

Todd, S.W., R.M. Hoffer and D.G. Milchunas. 1998. Biomass estimation on grazed and ungrazed rangelands using spectral indices. International Journal of Remote Sensing 19: 427-438.

Wachendorf, M. 1995. Untersuchungen zur Ertrags- und Qualitätsentwicklung von Rotklee und Rotkleegras in Abhängigkeit von der Nutzungsfrequenz, der Stickstoffdüngung und der Grasart. Ph.D. thesis Univ. of Kiel, Germany.

Wachendorf, M., M. Büchter, H. Trott and and F. Taube. 2004. Performance and environmental effects of forage production on sandy soils. II. Impact of defoliation system and nitrogen input on nitrate leaching losses. Grass and Forage Science 59: 56-68.

Watson, C.A., D. Atkinson, P. Gosling, L.R. Jackson and F.W. Rayns. 2002. Managing soil fertility in organic farming systems. Soil Use and Management 18: 239-247.

Weißbach, F. 1995: Über die Schätzung des Beitrages der symbiontischen N_2-Fixierung durch Weißklee zur Stickstoffbilanz von Grünlandflächen. Landbauforschung Völkenrode, 45 (2): 67-74.

Wilhelmy, B. 1993. Untersuchungen zur Ertragsbildung und zur Veränderung ausgewählter Qualitätsparameter im Zuwachsverlauf von Grünlandbeständen in Abhängigkeit von der botanischen Zusammensetzung (mit/ohne Weissklee), der Stickstoffdüngung und der Nutzungshäufigkeit. PhD. thesis Univ. of Kiel, Germany.

WinISI III. 2005. Manual, Version 1.60. Infrasoft International, LLC., State College, PA, USA.

Ye, X., K. Sakai, M. Manago, S. Asada and A. Sasao. 2007. Prediction of citrus yield from airborne hyperspectral imagery. Precision Agriculture 8: 111-125.

Zha, Y., J. Gao, S. Ni, Y. Liu, J. Jiangand Y. Wei. 2003. A spectral reflectance-based approach to quantification of grassland cover from Landsat TM imagery. Remote Sensing of Environment 87: 371-375.

Zhao, D., K.R. Reddy, V.G. Kakani, J.J. Read and G.A. Carter. 2003. Corn (*Zea mays* L.) growth, leaf pigment concentration, photosynthesis and leaf hyperspectral reflectance properties as affected by nitrogen supply. Plant and Soil 257: 205–217.

Zhao, D., K.R. Reddy, V.G. Kakani and V.R. Reddy. 2005. Selection of optimum reflectance ratios for estimating leaf nitrogen and chlorophyll concentrations of field-grown cotton. Agronomy Journal 97: 89-98.

Zwiggelaar, R. 1998. A review of spectral properties of plants and their potential use for crop/weed discrimination in row-crops. Crop protection 1: 189-206.