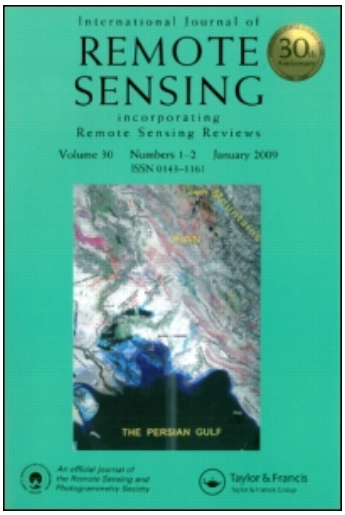

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Grass species differentiation through canopy hyperspectral reflectance

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This study attempts (1) to evaluate the capability of hyperspectral reflectance to differentiate C_3 and C_4 grass species, both in isolation and in mixed canopies; (2) to identify the critical spectral ranges that differentiate the two groups and individual species within them; and (3) to determine if there is temporal variation in these capabilities. During one year, hyperspectral reflectance of C_3 and C_4 grass species was measured both in single-species and in mixed canopies. Spectral bands with higher differentiating potential were identified and species classified. For single-species canopies, hyperspectral reflectance differentiated the two functional groups and most species in all seasons. In mixed canopies, it underestimated the fractional cover of the C_4 component. The green, red, and near infrared above 820 nm spectral ranges were critical both for species and functional group differentiation. In conclusion, hyperspectral information was useful to differentiate pure canopies, but the differentiation algorithms were season-specific. Additionally, we need to improve our understanding of interactive effects of species in order to accurately estimate the composition of assemblages.

1. Introduction

Temperate rangelands and pastures are often dominated by a variable proportion of cool-season and warm-season grasses (Ode *et al.* 1980, Lauenroth and Milchunas 1991, Soriano *et al.* 1992, Paruelo and Lauenroth, 1996), which correspond to the carbon 3 (C_3) and carbon 4 (C_4) photosynthetic syndrome (Taiz and Zeiger 1999). It is critical for both productive and scientific purposes to know the relative abundance in terms of cover, biomass or aboveground net primary production of these groups, e.g. characterization of forage quality and seasonality (Corson *et al.* 2007, Beeri *et al.* 2007) and evaluation of the impact of increasing atmospheric CO_2 on the amount and seasonality of net primary production (Wand *et al.* 1999, Ehleringer *et al.* 2002). For many large-scale objectives, field sampling of C_3–C_4 abundance is logistically impossible. Correlative models between plant functional type abundance and climatic variables have described regional patterns of C_3–C_4 abundance (Paruelo and Lauenroth 1996, Epstein *et al.* 2002), but these models cannot be used at landscape scale.

C_3 and C_4 species differ in many anatomical and physiological aspects. The main difference is that C_4 species have the phosphoenolpyruvate carboxylase enzyme, besides the ribulose-1.5-bisphosphate carboxylase/oxygenase enzyme, and a vascular bundle sheath where this enzyme is concentrated (Hatch and Osmond 1976, Pearcy and Ehleringer 1984). In addition to these two fundamental aspects, other

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morphological differences have been described (Dengler *et al.* 1994, Craine *et al.* 2001, 2005, Wright *et al.* 2004, Oyarzabal *et al.* 2008). Considering only grass species, both leaf area and specific leaf area ($cm^2\ g^{-1}$) are higher in C_4 than in C_3 species (Oyarzabal *et al.* 2008), while relative water content and nitrogen concentration are higher in C_3 species (Craine *et al.* 2005, Oyarzabal *et al.* 2008). Leaves of C_4 grasses have a more compact mesophyll, a higher proportion of vascular tissue and a lower interveinal distance than C_3 leaves (Dengler *et al.* 1994, Ogle 2003). The main physiological consequences of these morphological and biochemical differences are that C_3 species have lower water and nitrogen use efficiency than C_4 species, which results in a lower radiation use efficiency (Pearcy and Ehleringer 1984).

Anatomical and physiological differences such as those described for C_3 and C_4 grasses may be detected by leaf reflectance at narrow intervals of the electromagnetic spectrum (usually less than 10 nm), i.e. hyperspectral reflectance. Canopy reflectance depends on leaf surface properties, internal structure and biochemical component concentration (Peterson *et al.* 1988, Curran 1989, Peñuelas 1998). Considering a wide range of species, including C_3 and C_4 grasses, the content of pigments, nitrogen, carbon compounds (such as lignin) and water have been strongly and consistently related to spectral indices, based on plant reflectance at narrow intervals (Peterson *et al.* 1988, Curran 1989, Peñuelas *et al.* 1997, Curran *et al.* 2001, Sims and Gamon 2002 among others). For example, across species, leaf nitrogen content was best predicted through the combination of the reflectance at 1770 nm and 693 nm (Ferwerda *et al.* 2005). In winter wheat, a C_3 species, normalized spectral indices based on the red edge region (680–750 nm) were correlated with chlorophyll density (mg chlorophyll m^{-2} soil), and nitrogen density (g nitrogen m^{-2} soil), whereas other indices based on the blue part of the spectrum were correlated with the concentration of chlorophyll and nitrogen, mg g^{-1} leaf fresh weight (Hansen and Schjoerring 2003). In C_4 species, macronutrient concentration was predicted by hyperspectral information in the visible portion (550–750 nm), and in the shortwave infrared (1634–1786, 2006–2196 nm) one (Mutanga *et al.* 2004). If the variation of these traits within each photosynthetic group was detected by hyperspectral reflectance, using this method to discriminate between groups appears promising. Finally, the reflectance in the 531 nm band proposed as an indicator of radiation use efficiency (Gamon *et al.* 1997) appears as a narrow but sensitive portion of the spectrum, capable of discriminating among C_3 and C_4 species, which, as indicated above, widely differ in said efficiency.

Hyperspectral reflectance has been evaluated as a tool to detect differences in vegetation community attributes, such as the composition of species and plant functional types, obtaining different degrees of success and applying increasing complexity of data analysis. Initially, the studies were based on univariate, parametric analysis. Although grass and woody tissues were differentiated (Asner 1998), these types of studies were unable to discriminate between C_3 and C_4 species (Asner *et al.* 1998) or among leaves of 26 species across a wide range of life forms, from annual herbs to trees (Knapp and Carter 1998). Studies based on non-parametric, univiariate analysis have been more successful: Schmidt and Skidmore (2003) discriminated 27 saltmarsh vegetation associations, mainly composed of grasses and sedges, and Schmidt and Skidmore (2001) differentiated eight monoculture C_4 grass canopies under laboratory conditions. Finally, multivariate techniques applied to differentiate species have detected a weed, *Ipomoea lacunosa L.*, in soybean crops, although the critical bands differed depending on weed phenology (Koger *et al.* 2003), and served to discriminate liana from tree leaves, which was apparently due to their different chlorophyll concentration, particularly under drought conditions (Castro-Esau *et al.* 2004).

Although these results show the potential of hyperspectral reflectance to differentiate vegetation entities in complex canopies (i.e. species or plant functional type composition), some unexplored issues have been identified. First, no study has succeeded in detecting C_3 and C_4 pure canopies or estimating the relative abundance of each group in mixtures by hyperspectral reflectance. Second, it is unknown which portions of the electromagnetic spectrum are more relevant in discriminating these grass species or groups. Third, the capability to differentiate species in different phenological stages, a problem highlighted by the results on *Ipomoea lacunosa* described above, remains unknown. Thus, the objectives of this work were (1) to assess the capabilities of hyperspectral sensors to distinguish C_3 and C_4 grass species from temperate grasslands, both in isolation and in mixtures, (2) to identify the spectral bands that are most relevant to differentiate grass species, and (3) to determine if plant phenology modifies the ability of hyperspectral data to discriminate among species.

2. Methods

2.1 *Experimental conditions*

In November 2001, equal number of tillers from adult plants of *Festuca ovina* var. *glauca* L., *Festuca arundinacea* Schreb., *Phalaris aquatica* L., *Dactilys glomerata* L. (all C_3), *Paspalum dilatatum* Poir., *Cynodon dactylon* (L.) Pers., and *Axonopus suffultus* (Mikan) Parodi (all C_4) were placed in 3000 cm^3 plastic pots filled with washed sand. Plants were grown in a common garden in the Faculty of Agronomy, University of Buenos Aires, Buenos Aires, Argentina (34°35′ S, 58°29′ W). Pots with each species (number of pots, $n = 54$) were arranged in 6×9 pot rectangles. Perimeter pots were not used to avoid edge effects. We allowed a 10-month acclimation period to homogenize plant condition. Nutrients were added every other month at a rate of 2 g/pot of fertilizer throughout the experiment (grade: N 15%, P 2.2%, K 29%). Water was added daily until drainage from the pots was observed. During the experiment period, May 2002 to August 2003, minimum average monthly temperature was 10.7°C (June 2002) and maximum average monthly temperature was 25.8°C (January 2003).

2.2 *Data acquisition*

Hyperspectral measurements were carried out during August (winter), November (spring), January (summer), and May (fall) during the 2002–2003 growing season. In November, *Axonopus suffultus* was not considered due to technical problems with the sensor. Hyperspectral reflectance data were collected with a hand-held Fieldspec spectrometer ASD (FieldSpec® Handheld**,** 325–1075 nm resolution; Analytical Spectral Devices, Inc., Boulder, CO, USA). This spectrometer registers radiation at 1.4 nm intervals across the visible/near-infrared region of the spectrum using a fibre optic cable with a 25° field of view. Reflectance was calculated as the ratio between the reflected energy from the target (i.e. canopy) and the incident energy on the barium sulfate (BaSO4) white reference.

In order to avoid external light influences, reflectance was collected under constant light conditions. The pots to be measured were placed in a black wooden box, 150 cm high, 60 cm wide, illuminated with three 150 W tungsten lamps (Philips Spotline R95, Buenos Aires, Argentina) which were inside the box, 125 cm above the canopy (figure 1). The fibre optic cable was positioned at a height of 120 cm at nadir (figure 1). On each

measurement date, the hyperspectral signals of single-species and mixed canopies were collected. For single-species canopies, 27 pots were randomly selected and three 3 × 3 pot canopies per species were constructed, and sequentially placed inside the wooden box, where their hyperspectral signal was collected (figure 1: seven species × three canopies = total 21 canopies per date). For mixed canopies, two species, *Festuca ovina* var. *glauca* (C_3) and *Cynodon dactylon* (C_4) were mixed in three types of arrangements (figure 2). The first type considered either six pots of the C_3 species and three of the C_4 species or six pots of the C_4 species and three pots of the C_3 species (figure 2). The six pots with the same species were together in a 2 × 3 arrangement and the three pots with the other species were next to the former in a 1 × 3 arrangement (figure 2). In the third type of spatial arrangement, five pots of the C_4 species were located in the two diagonals of the 3 × 3 pot matrix, and four pots of the C_3 occupied the remaining positions (figure 2).

The central plant of each 3 × 3 arrangement of each single-species canopy was harvested on each measurement date. Its biomass was separated into photosynthetic and non-photosynthetic compartments, and photosynthetic area (cm^2) was determined by a LICOR area meter (LI-3100 Area Meter). All compartments were dried at 70°C for a 72-h period and then weighed. This information allowed estimation of the photosynthetic and non-photosynthetic biomass and area per canopy and its ratio, specific photosynthetic area ($m^2\ kg^{-1}$).

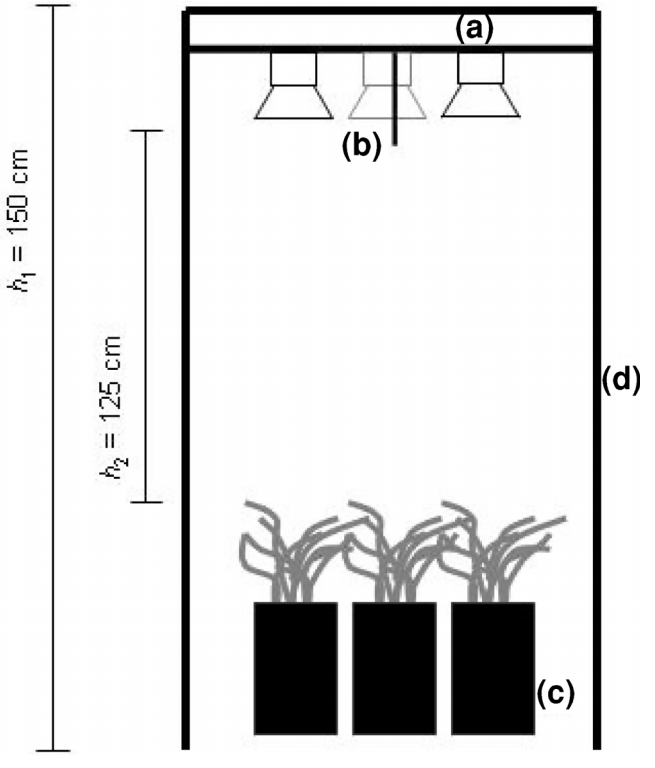

Figure 1. Schematic diagram of the hyperspectral spectrum measurements under constant light conditions, where (a) = lamps, (b) = sensor, (c) = canopy and (d) = black wooden box. h_1 represents the total height of the box and h_2 represents the distance between the sensor and the top of the pots.

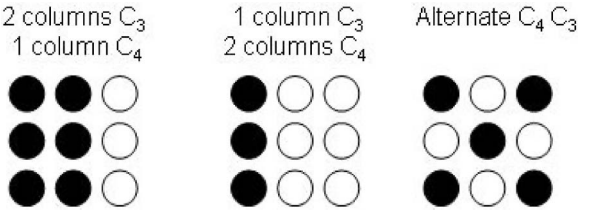

Figure 2. Schematic diagram of the mixed canopies arrangement. Empty circles represent *Cynodon dactylon* pots (C_4 species) and black circles represent *Festuca glauca* pots (C_3 species).

2.3 *Data analysis*

Different methodologies have been applied to distinguish species based on hyperspectral data, which suggests that there is not yet a standard technique. For example, the four papers more closely related to our objective used different analytical approaches (Thenkabail *et al.* 2000, Koger *et al.* 2003, Schmidt and Skidmore 2003, Castro-Esau *et al.* 2004). However, three common steps were identified in all methods: (1) data transformation; (2) feature extraction; and (3) classification algorithms. Thus our methodology will be described in terms of these three steps.

2.3.1 Data transformation. Data transformation involved smoothing, to avoid noise associated with specific bands; and normalization, to isolate a particular absorption feature from a changing background. We smoothed data by averaging two 1.4 nm intervals, which will be called 'bands' hereafter, and normalized them by dividing each band by the maximum band value of each spectrum. Since both extremes of the reflectance spectrum were discarded due to noise, the final spectrum considered in the analysis had 183 2.8 nm bands and an effective working region of 450 to 1000 nm.

2.3.2 Feature extraction. Because hyperspectral sensors provide high dimensional and often highly correlated data, it is usually necessary to reduce data dimensionality to retrieve reliable multivariate statistics (e.g. Koger *et al.* 2003). Consequently, principal component analysis (PCA) over the smoothed spectra was performed. In PCA, a p-dimensional data space (the waveband spectral bands) is represented as a set of mutually orthogonal ordination axes (Kenkel *et al.* 2002). The PCA gives as a result a list of axes, the variance being explained by each of them, as well as the importance of each band within each axis. Thus, the importance of each band in discriminating species was quantified as a band relative importance (BRI) score as shown in equation (1):

$$\mathrm{BRI}_i = \sum_{j=1}^{n} \mathrm{e}_j \times |\mathrm{w}_{ij}| \tag{1}$$

where BRI_i is the relative importance of band i to band n, e_j represents the percentage of variance explained by axis j and $|w_{ij}|$ is the absolute weight of band i in the axis j. Only the axes above the 'broken-stick' eigenvalue were included (Legendre and Legendre 1998). Therefore, BRI expresses the overall relative importance of each spectral band.

2.3.3 Classification of species and functional types. For each measurement season, ANOVAs were performed to evaluate the differences among species and functional types (C_3 and C_4 species) based on hyperspectral signals and spectral indices. The response variables were the PCA scores from axes I and II per species or functional group with 2.8 nm spectral resolution (Craine *et al.* 2001) and two generic, broader spectral indices: the ratio between two bands, i.e. band *a*/band *b*, and a normalized index (band *a* – band *b*)/(band *a* + band *b*). The total number of bands to calculate these broader indices was six. Each bandwidth was selected based on LANDSAT spectral ranges and it represented the reflectance average value across the spectral range: band 1 452–522 nm, band 2 525–601 nm, band 3 605–627 nm, band 4 630–690 nm, band 5 760–900 nm, and band 6 903–999 nm. The number of replicates was three per species, nine for the C_4 group (six in spring when *Axonopus suffultus* spectra were not considered), and 12 for the C_3 group. To evaluate if canopy structural variables were related with differences among species and functional type spectral responses, correlations between spectral data (PCA axes, spectral indices) and canopy structural variables (photosynthetic and non photosynthetic biomass, photosynthetic area, photosynthetic and non photosynthetic ratio and specific leaf area) were calculated.

2.3.4 C_3/C_4 species abundance. Linear spectral mixture analysis linearly estimates the proportion of individual identities, in this case species, from the mixing of their reflectance spectra (Wessman *et al.* 1997, Clark 1999, Bateson *et al.* 2000, Dennison and Roberts 2003). This technique was applied to evaluate mixed canopies. The endmembers were the average hyperspectral spectra of pure *Cynodon dactylon* and *Festuca ovina* var. *glauca* canopies. Each spatial arrangement of mixed canopies (figure 2) had an observed proportion of C_3 and C_4 cover, which was calculated as the number of pots per species multiplied by the average photosynthetic area per pot and species. Additionally, each spatial arrangement had an observed hyperspectral spectrum. By linear spectral mixture analysis, all the potential hyperspectral spectra between 1 and 99% cover of each endmember was generated. Finally, a PCA was performed in order to assign each observed hyperspectral spectrum to the closest spectrum of those generated by linear spectral mixture analysis. The proximity measurement was the minimum Euclidean distance. The C_3/C_4 proportion predicted was then compared to the observed proportion based on photosynthetic area by linear regression.

3. Results

Hyperspectral analysis successfully differentiated functional types and species in all seasons (figure 3). In winter, the two photosynthetic groups significantly differed across axis I due to the response of *C. dactylon* and *P. dilatatum* (figure 3). Of the differences among species, 29% were based on their position on axis I of the PCA analysis, 14% on axis II, and 57% on both axes (figure 3 and Table 1, winter). In spring, the two photosynthetic groups significantly differed across PCA axis II due to the response of *C. dactylon* and *P. dilatatum* (figure 3). Of the significant differences among species, 31% were based on their position on axis I of the PCA analysis, 31% on axis II, and 38% on both axes (figure 3 and Table 1, spring). In summer, the two photosynthetic groups significantly differed across both PCA axes (figure 3). Of the differences among species, 64% were based on their position on axis I of the PCA analysis, 21% on axis II, and 14% on both axes (figure 3 and Table 2, summer). In

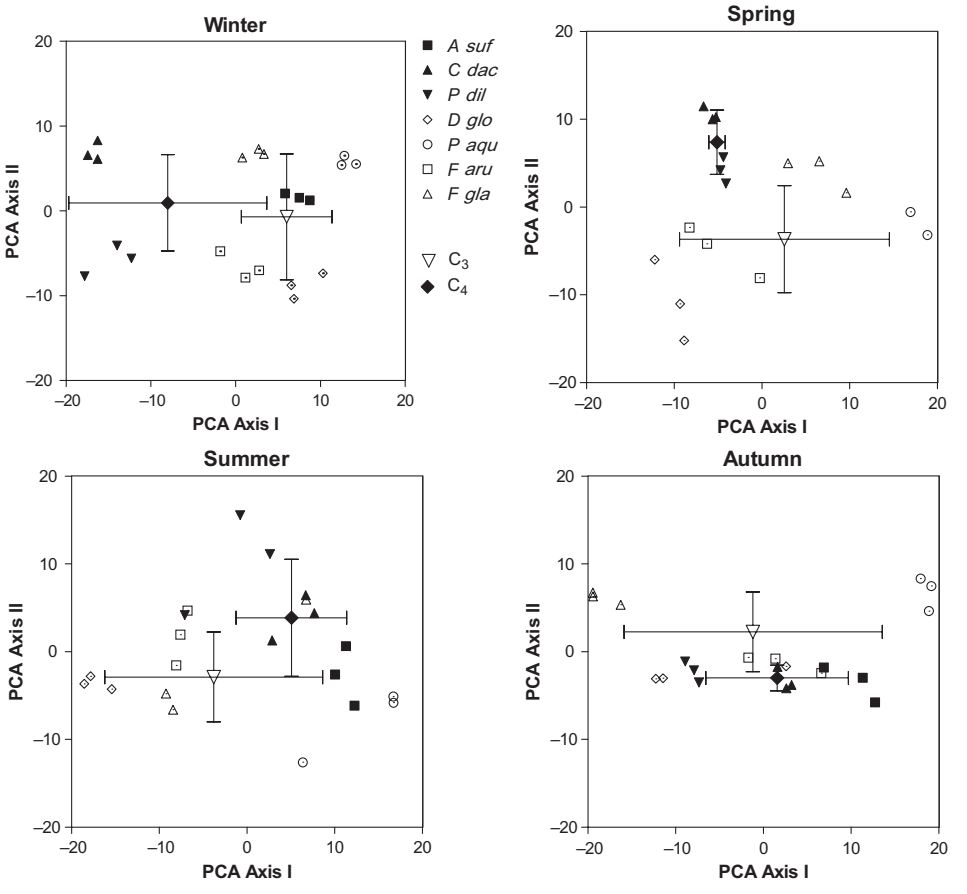

Figure 3. Position of the different species in two synthetic spectral dimensions based on the 450–1000 nm hyperspectral range, through PCA multivariate analysis. *A suf*: *Axonopus suffultus, C dac*: *Cynodon dactylon,* and *P dil*: *Paspalum dilatatum* (C_4) species*, D glo*: *Dactilys glomerata, P aqu*: *Phalaris aquatica, F aru: Festuca arundinacea,* and *F gla*: *Festuca glauca* (C_3) species. Additionally, the empty, inverted triangle and the black diamond represent the average score for C_3 and C_4 groups respectively. Error bars represent standard deviation.

autumn, the two photosynthetic groups significantly differed across PCA axis II (figure 3). Of the differences among species, 24% were based on their position on PCA axis I, 15% on axis II, and 62% on both axes (figure 3 and Table 2, autumn). Summarizing these hyperspectral analyses, 79.5% of the total number of pairs of species significantly differed (62 out of 78, $p < 0.05$; figure 3 and Tables 1 and 2).

Spectral ranges sensible for functional types and species differentiation varied among seasons (figure 4). In winter, based on absolute maximum eigenvector values, the most sensitive reflectance ranges on axis I were 598–703 and 808–881 nm (figure 4). Differences along axis II were largely a consequence of reflectance in the 722–760 nm range (figure 4). In the cases where differences occurred in both axes, BRI analysis showed the highest values in the 559–560 nm range (figure 4). In spring, PCA axis I was a contrast between the reflectance in the 500–547 and 884–932 nm ranges (figure 4). On axis II, differences among species were largely a consequence of reflectance in the 640–671 and 859–868 nm

Table 1. Hyperspectral reflectance significant differences between all species pairs ($p < 0.05$) in winter (lower-left half of the table) and spring (upper-right half of the table). The numbers in each cell indicate the PCA axes along which the corresponding species pair showed significant differences. The symbol — represents no data and empty cells indicate lack of significant difference.

	A suf	*C dac*	*D glo*	*P bul*	*F aru*	*F gla*	*P dil*
A suf		—	—	—	—	—	—
C dac	I–II		II	I–II	II	I	
D glo	II	I–II		I–II		I–II	II
P bul	I–II	I	I–II		I	I	I–II
F aru	I–II	I–II	I	I–II		I–II	II
F gla	I–II	I	I–II	I	II		I
P dil	I–II	II	I	I–II	I	I–II	

A suf: *Axonopus suffultus*, *C dac*: *Cynodon dactylon* and *P dil*: *Paspalum dilatatum* (C_4). *D glo*: *Dactilys glomerata*, *P aqu*: *Phalaris aquatica*, *F aru: Festuca arundinacea*, and *F gla*: *Festuca glauca* (C_3).

Table 2. Hyperspectral reflectance significant differences between all species pairs ($p < 0.05$) in summer (lower-left half of the table) and autumn (upper-right half of the table). The numbers in each cell indicate the PCA axes along which the corresponding species pair showed significant differences. Empty cells indicate lack of significant difference.

	A suf	*C dac*	*D glo*	*P bul*	*F aru*	*F gla*	*P dil*
A suf			I	II		I–II	I
C dac				I–II		I–II	
D glo	I	I		I–II		I–II	
P bul		II	I		I–II	I	I–II
F aru	I	I		I		I–II	
F gla	I		I	I			II
P dil	II		I–II	I–II		II	

For abbreviations see table 1.

ranges (figure 4). Differences on both axes based on the BRI analysis showed the highest values in the 570–576 nm range (figure 4). In summer, PCA axis I differences among species were largely a consequence of the reflectance in the 528–976 nm range, particularly between 716 and 729 nm (figure 4). On axis II, differences among species were largely a consequence of reflectance in the 452–640 nm range, particularly between 484 and 513 nm (figure 4). Differences on both axes based on the BRI analysis showed the highest values in the 554–621 and 852–929 nm ranges (figure 4). In autumn, PCA axis I was largely a consequence of the reflectance in the 573–706 nm range, particularly between 681 and 700 nm, and in the 862–910 nm range (figure 4). On axis II, differences among species were a consequence of hyperspectral properties in the 725–729 and 967–999 nm ranges (figure 4). Differences on both axes based on the BRI analysis showed the highest values in the 849–868 nm range (figure 4).

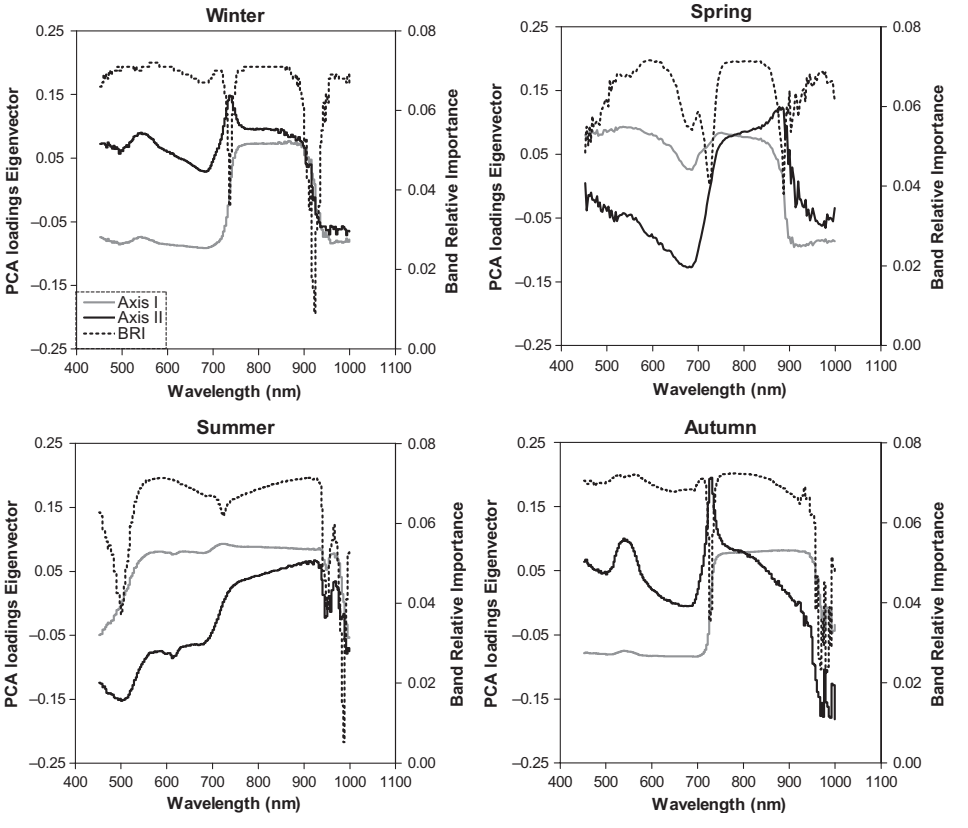

Figure 4. PCA loadings (eigenvector) and BRI, band relative importance, per measurement season and wavelength. PCA loadings indicate the relative importance of each spectral band in each PCA axis. BRI indicates the relative importance of the spectral bands considering both PCA axes.

Spectral indices also differentiated functional types (figure 5), and species (figure 6). In all seasons, except in autumn when spectral indices failed to differentiate functional types, a certain combination of bands was more sensitive than PCA analysis (figure 5). The indices with more differentiation ability were based on bands 1 and 3 in winter, 3 and 5 in spring, and 1 and 5 in summer (figure 5). In general, normalized-difference and ratio indices performed similarly.

The proportion of significantly different interspecific comparisons between pairs of species, found by hyperspectral analysis, declined from winter to autumn (figure 6). Spectral indices were not as able to differentiate species as hyperspectral analysis (figure 6). None of the spectral indices differentiated all pair of species in each season. The average proportion of significantly different interspecific comparisons also declined from winter to summer, but increased in autumn (figure 6). The most successful indices (data not shown) were as follows: (a) In winter, the normalized difference of bands 1 and 4, and 2 and 3, and ratio of the latter (proportion of significantly different pairs of species = 90%); (b) in spring, the normalized difference and ratio between bands 5 and 6 (78%); (c) in summer, the normalized difference between bands 2 and 6 (67%); (d) in autumn, the normalized difference between bands 1 and 3 (71%).

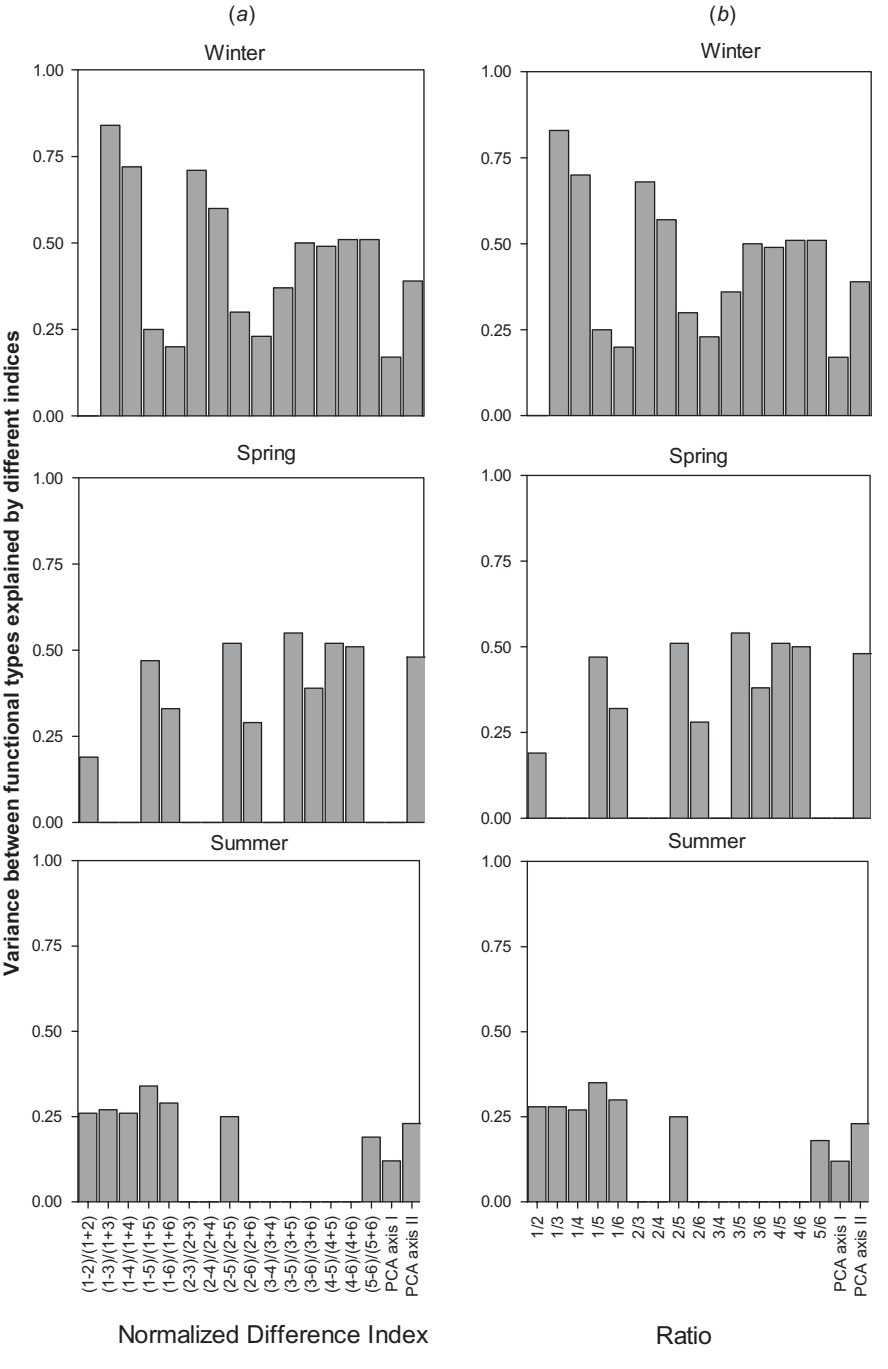

Figure 5. Functional types, C_3-C_4, variance proportion explained by each spectral index and PCA axes in three seasons. (*a*) Normalized spectral indices: (band *a* – band *b*)/(band *a* + band *b*). (*b*) Ratio indices: band *a*/band *b*. Only statistically significant analyses ($p < 0.05$) are reported. The spectral indices were generated as the combination of six different bands, similar to LANDSAT ranges. Band 1 452–522 nm, band 2 525–601 nm, band 3 605–627 nm, band 4 630–690 nm, band 5 760–900 nm and band 6 903–999 nm. Autumn was not included in the figure because no index differentiated between functional types.

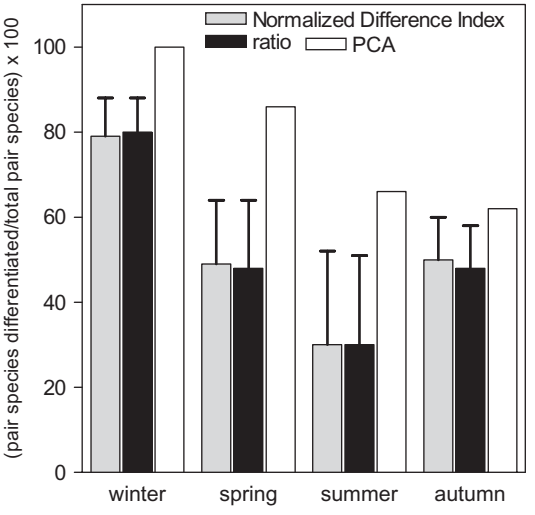

Figure 6. Average percentage of significantly differentiated pairs of species per season and differentiation tool. The error bars represent the standard deviation. Each value was obtained as the ratio between the total number of significantly differentiated pairs of species and the total number of pairs of species.

Except in spring, the various canopy structural variables evaluated (Table 3) were unrelated to the differences among species, as evidenced by a correlation analysis between each of these variables and the scores of species on PCA axes, or the spectral indices with the maximum percentage of significantly different pairs of species. In spring, there were significant correlations between (a) photosynthetic biomass, expressed in g m^{-2}, and the normalized difference index (R: 0.84, $p < 0.05$) or the scores on PCA axis II (R: 0.85, $p < 0.05$), and (b) the photosynthetic/non photosynthetic biomass ratio and scores on PCA axis I (R: 0.95, $p < 0.05$).

Compared to single-species canopies, composition of mixed canopies (fractional cover of C_3 and C_4 species) was less successfully estimated by hyperspectral reflectance (figure 7). Linear spectral mixture analysis tended to underestimate the fractional cover of the C_4 component (figure 7), particularly on two dates, when the observed fraction of this group was very high (spring and summer). However, excluding these cases, the average error in predicting fractional cover was 15%.

4. Discussion

Our results showed that (1) functional types may be differentiated in single-species canopies by both hyperspectral analysis and spectral indices; (2) individual species within functional types may also be differentiated by hyperspectral analysis and, with lower accuracy, by broader spectral indices; (3) composition of mixed canopies is more difficult to estimate, particularly when a large proportion of one group is observed; (4) differentiation capability changes with seasons; and (5) spectral ranges necessary for differentiation are relatively wide and change among seasons and species. To the best of our knowledge, there is no evidence found in literature of changes in the capability to differentiate single-species grass canopies according to their phenological status. With different accuracy across seasons, we distinguished

Table 3. Canopy structural variables per species and seasons.

Species	NP biomass ($g\ m^{-2}$)	P biomass ($g\ m^{-2}$)	P/NP	LPA(cm^2)	Specific leaf area ($m^2\ kg^{-1}$)	LAI
Winter						
A suf	101.52	1002.09	9.87	894	3.51	3.51
C dac	33.40	784.64	23.49	341	1.71	1.34
P dil	383.81	104.14	0.27	311	11.75	1.22
D glo	332.06	258.71	0.78	308	4.68	1.21
P bul	244.95	246.92	1.01	709	11.28	2.79
F aru	354.33	349.75	0.99	392	4.41	1.54
F gla	297.35	203.04	0.68	289	5.60	1.14
Spring						
A suf						
C dac	1045.31	347.13	0.33	745	8.44	2.93
P dil	492.53	343.20	0.70	481	5.50	1.89
D glo	297.35	87.76	0.30	334	14.95	1.31
P bul	174.22	323.81	1.86	627	7.61	2.46
F aru	445.37	229.24	0.51	701	12.01	2.75
F gla	417.86	313.07	0.75	737	9.25	2.90
Summer						
A suf	941.04	405.55	0.43	1018	9.86	4.00
C dac	407.52	992.66	2.44	958	3.79	3.77
P dil	558.94	175.00	0.31	341	7.66	1.34
D glo	498.82	55.28	0.11	192	13.63	0.75
P bul	358.52	136.89	0.38	247	7.08	0.97
F aru	296.43	66.54	0.22	179	10.56	0.70
F gla	365.07	43.49	0.12	90	8.12	0.35
Autumn						
A suf	1005.10	672.77	0.67	1187	6.93	4.66
C dac	522.66	779.40	1.49	977	4.93	3.84
P dil	167.67	56.33	0.34	214	14.90	0.84
D glo	104.79	73.36	0.70	816	43.70	3.21
P bul	133.61	155.88	1.17	879	22.16	3.45
F aru	171.60	94.31	0.55	169	7.06	0.67
F gla	282.94	82.13	0.29	160	7.66	0.63

NP = non photosynthetic, P = photosynthetic, LPA = leaf photosynthetic area, LAI = Leaf Area Index. Species: *A suf*: *Axonopus suffultus*, *C dac*: *Cynodon dactylon*, and *P dil*: *Paspalum dilatatum* (C_4). *D glo*: *Dactilys glomerata*, *P aqu*: *Phalaris aquatica*, *F aru: Festuca arundinacea*, and *F gla*: *Festuca glauca* (C_3).

species differing in photosynthetic syndrome (C_3 and C_4) and individual species within these groups (figure 3).

Differentiation was lower in summer and autumn than in winter and spring (figure 3), when the relative proportion of live tissue was lower (Table 3), as in rangelands and pastures (Sala *et al.* 1981, Oesterheld and León 1987). It is likely that less photosynthetic tissue, and lower organic compound concentration, were responsible for this lower variability in spectral responses (Sims and Gamon 2002, Mutanga *et al.* 2003, Hansen and Schjoerring 2003), and, consequently, there is less probability to differentiate species. The lack of correlation between spectral discrimination and number of canopy structural variables (Table 3) suggests that species and functional types were discriminated by intrinsic leaf properties, such as pigment composition and content, or mesophyll structure.

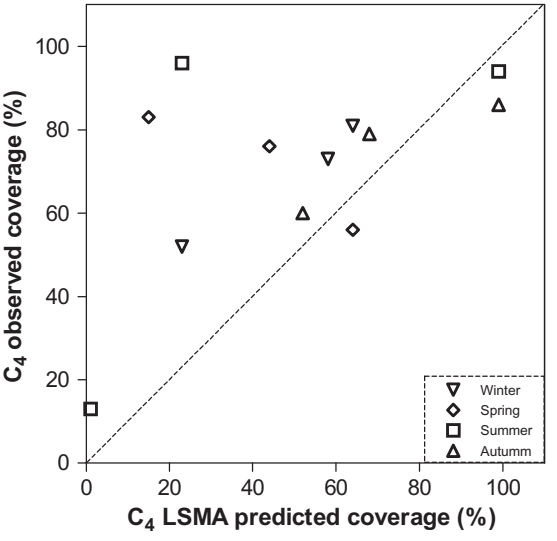

Figure 7. Observed and predicted cover of *Cynodon dactylon*, C_4, in mixtures with *Festuca glauca*, C_3, for different seasons. Observed values are based on leaf area data, whereas predicted values are based on linear spectral mixture analysis (LSMA).

Spectral indices, PCA eigenvectors and BRI analysis showed that relatively large ranges of bands would have been similarly successful in discriminating between both isolated species and the two functional groups (figures 4 and 5), but, nonetheless, narrow waveband ranges were particularly valuable and differed among seasons. These narrow waveband ranges were principally concentrated in the green and red portion of the visible part and the red edge portion of the spectrum (figure 4). Some of these narrow wavebands concurred with critical bands considered in other studies. Particularly, in the red portion of the spectrum the interaction with chlorophyll content plays a major role in leaf optical signals (Lichtenthaler 1987, Lichtenthaler and Buschmann 2001, Sims and Gamon 2002), suggesting its importance in the different response of functional groups and species. At low chlorophyll concentrations, reflectance sensitivity is higher around 680 nm, whereas at medium to high concentrations, reflectance sensitivity is higher at 550 nm (Jacquemoud and Baret 1990, Peñuelas 1998). In winter, spring and summer, BRI analysis revealed that ranges close to 550 nm were critical (figure 4), which suggests the importance of chlorophyll for functional groups and species differentiation based on both PCA axes. The red edge region (Peñuelas 1998) close to the 750 nm was also critical (figure 4). In this range, brown pigments become important in leaf optical signals (Peñuelas 1998). A range around 750 nm was particularly important in summer and on axis I (figure 4). Previous works have identified critical bands within the visible part of spectrum and the near infrared portion above 1200 nm (Schmidt and Skidmore 2003, Mutanga *et al.* 2004, Thenkabail *et al.* 2000). In spring and autumn critical bands corresponded to the near infrared portion of the spectrum, between 700 and 1000 nm (figure 4), a portion that had previously proven critical only by Thenkabail *et al.* (2000).

Mixed canopies introduce the additional challenge of interactive effects (Peterson *et al.* 1988, Wessman *et al.* 1997). Paradoxically, the two situations in which linear spectral mixture analysis clearly failed had an extremely large proportion of the C_4

component, which, *a priori*, would be the easiest to estimate, based on isolated species signatures. These two cases were just one end of a general trend to underestimate the C_4 component. The mixed canopies were composed by a prostrate C_4 species and an erect C_3 species. Thus, it is likely that reflectance of the erect C_3 species was over-represented, and this overrepresentation was more marked in the months with large biomass of the C_3 species (Gamon *et al.* 1997).

5. Conclusions

Our results indicate that hyperspectral analysis and spectral indices based on widely used bands may differentiate single-species forage grass canopies by functional type and species. It has been shown that differentiation capability depends on the season and will require ad-hoc calibrations to select a specific model and set of bands. Predicting the composition of mixed canopies seems to be influenced by interactive effects, particularly when species have different canopy architecture. In the field, pure canopies are expected to occur in monophytic pastures or in those strongly dominated by a single species, which could be readily assessed by this technique. However, the 'pure' or 'mixed' nature of the problem will depend on image resolution and pasture structure. More research is needed to understand the spectral behaviour of complex canopies formed by many species with different architecture, and the background effects of different soil types (Singh and Sirohi 1994). Radiation transfer models (Verhoef 1984, Jacquemoud and Baret 1990, Jacquemoud *et al.* 2000) may help to understand the nature of the interaction of the optical properties of different leaves and their geometrical distribution in the canopy.

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References

Asner, G.P., 1998, Biophysical and biochemical sources of variability in canopy reflectance. *Remote Sensing of Environment*, **64**, pp. 234–253.

Asner, G.P., Wessman C.A. and Archer, S., 1998, Scale dependence of absorption of photosynthetically active radiation in terrestrial ecosystems. *Ecological Applications*, **8**, pp. 1003–1021.

Bateson, C.A., Asner, G.P. and Wessman, C.A., 2000, Endmember bundles: a new approach to incorporating endmember variability into spectral mixture analysis. *IEEE Transactions on Geoscience and Remote Sensing*, **38**, pp. 1083–1094.

Beeri, O., Phillips, R., Hendrickson, J., Frank, A.B. and Kronberg, S., 2007, Estimating forage quantity and quality using aerial hyperspectral imagery for northern mixed-grass prairie. *Remote Sensing of Environment*, **110**, pp. 216–225.

Castro-Esau, K.L.C., Sánchez-Azofeifa, G.A. and Caelli, T., 2004, Discrimination of lianas and trees with leaf-level hyperspectral data. *Remote Sensing of Environment*, **90**, pp. 353–372.

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

Corson, M.S., Rotz, C.A. and Skinner, R.H., 2007, Evaluating warm-season grass production in temperate-region pastures: A simulation approach. *Agricultural Systems*, **93**, pp. 252–268.

Craine, J.M., Froehle, J., Tilman, D.G., Wedin, D.A. and Chapin III F.S., 2001, The relationships among root and leaf traits of 76 grassland species and relative abundance along fertility and disturbance gradients. *Oikos*, **93**, pp. 274–285.

Craine, J.M., Lee, W.G., Bond, W.J., Williams, R.J. and Johnson, L.C., 2005, Environmental constraints on a global relationship among leaf and root traits of grasses. *Ecology*, **86**, pp. 12–19.

Curran, P.J., 1989, Remote sensing of foliar chemistry. *Remote Sensing of Environment*, **30**, pp. 271–278.

Curran, P.J., Dungan, J.L. and Peterson, D.L., 2001, Estimating the foliar biochemical concentration of leaves with reflectance spectrometry: Testing Kokaly and Clark methodologies. *Remote Sensing of Environment*, **76**, pp. 349–359.

Dengler, N.G., Dengler, R.E., Donnelly, P.M. and Hattersley, P.W., 1994, Quantitative leaf anatomy of C_3 and C_4 grasses (*Poaceae*) – Bundle sheath and mesophyll surface area relationships. *Annals of Botany*, **73**, pp. 241–255.

Dennison, P.E. and Roberts, D.A., 2003, Endmember selection for multiple endmember spectral mixture analysis using endmember average RMSE. *Remote Sensing of Environment*, **87**, pp. 123–135.

Ehleringer, J.R., Cerling, T.E. and Dearing, M.D., 2002, Atmospheric CO_2 as a global change driver influencing plant-animal interactions. *Integrative and Comparative Biology*, **42**, pp. 424–430.

Epstein, H.E., Gill, R.A., Paruelo, J.M., Lauenroth, W.K., Jia, G.J. and Burke, I.C., 2002, The relative abundance of three plant functional types in temperate grasslands and shrublands of North and South America: effects of projected climate change. *Journal of Biogeography*, **29**, pp. 875–888.

Ferwerda, J.G., Skidmore, A.K. and Mutanga, O., 2005, Nitrogen detection with hyperspectral normalized ratio indices across multiple plant species. *International Journal of Remote Sensing*, **26**, pp. 4083–4095.

Gamon, J.A., Serrano, L. and Surfus, J.S, 1997, The photochemical reflectance index: an optical indicator of photosynthetic radiation use efficiency across species, functional types, and nutrient levels. *Oecologia*, **112**, pp. 492–501.

Hansen, P.M. and Schjoerring J.K., 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**, pp. 542–553.

Hatch, M.D. and Osmond, C.B., 1976, Compartmentation and transport in C4 photosynthesis. In *Encyclopaedia of Plant Physiology*, C.R. Stocking and U. Heber (Eds.), pp. 144–184 (New York: Springer-Verlag).

Jacquemoud, S. and Baret, F., 1990, PROSPECT: A model of leaf optical properties spectra. *Remote Sensing of Environment*, **34**, pp. 75–91.

Jacquemoud, S., Bacour C., Poilvé, H. and Frangi, J.-P., 2000, Comparison of four radiative transfer models to simulate plant canopies reflectance: direct and inverse mode. *Remote Sensing of Environment*, **74**, pp. 471–481.

Kenkel, N.C., Derksen, D.A., Thomas, A.G. and Watson, P.R., 2002, Multivariate analysis in weed science research. *Weed Science*, **50**, pp. 281–292.

Knapp, A.K. and Carter, G.A., 1998, Variability in leaf optical properties among 26 species from a broad range of habitats. *American Journal of Botany*, **85**, pp. 940–946.

Koger, C.H., Bruce, L.M., Shaw, D.R. and Reddy, K.N., 2003, Wavelet analysis of hyperspectral reflectance data for detecting pitted morninglory (*Ipomoea lacunosa*) in soybean (*Glycine max*). *Remote Sensing of Environment*, **86**, pp. 108–119.

Lauenroth, W.K. and Milchunas, D.G., 1991, Short-Grass steppe. In *Natural Grasslands Introduction and Western hemisphere*, R.T. Coupland (Ed.), pp. 183–226. (Amsterdam: Elsevier).

Legendre, P. and Legendre, L., 1998, *Numerical Ecology* (Amsterdam: Elsevier).

Lichtenthaler, H.K., 1987, Chlorophylls and carotenoids: pigments of photosynthetic biomembranes. *Methods in Enzymology*, **148**, pp. 350–382.

Lichtenthaler, H.K., and Buschmann, C., 2001, Chlorophylls and carotenoids: measurement and characterization by UV-VIS spectroscopy. In *Current Protocols in Food Analytical Chemistry*, pp. 431–438 (New York: John Wiley).

Mutanga, O., Skidmore, A.K. and Van Wieren, S., 2003, Discriminating tropical grass (*Cenchrus ciliaris*) canopies grown under different treatments using spectroradiometry. *Photogrammetry and Remote Sensing*, **57**, pp. 263–272.

Mutanga, O., Skidmore, A.K. and Prins, H.H.T., 2004, Predicting *in situ* pasture quality in the Kruger National Park, South Africa, using continuum-removed absorption features. *Remote Sensing of Environment*, **89**, pp. 393–408.

Ode, D.J., Tieszen, L. and Lerman, J.C., 1980, The seasonal contribution of C_3 and C_4 plant species to primary production in a mixed prairie. *Ecology*, **6**, pp. 1304–1311.

Oesterheld, M. and León, R.J.C., 1987, El envejecimiento de las pasturas implantadas: su efecto sobre la productividad primaria. *Turrialba*, **37**, pp. 29–35.

Ogle, K., 2003, Implications of interveinal distance for quantum yield in C_4 grasses: a modeling and meta-analysis. *Oecologia*, **136**, pp. 532–542.

Oyarzabal, M., Paruelo, J.M., del Pino, F., Oesterheld, M. and Lauenroth, W.K., 2008, Trait differences between grass species along a climatic gradient in South and North America. *Journal of Vegetation Science*, **19**, pp. 183–192.

Paruelo, J.M. and Lauenroth, W.K., 1996, Relative abundance of plant functional types in grasslands and shrublands of North America. *Ecological Applications*, **6**, pp. 1212–1224.

Pearcy, R.W. and Ehleringer, J., 1984, Comparative ecophysiology of C_3 and C_4 plants. *Plant, Cell and Environment*, **7**, pp. 1–13.

Peñuelas, J., 1998. Visible and near-infrared reflectance techniques for diagnosing plant physiological status. *Trends in Plant Science*, **3**, pp. 151–155.

Peñuelas, J., Piñol, J., Ogaya, R. and Filella, I., 1997, Estimation of plant water concentration by the reflectance Water Index WI (R900/R970). *International Journal of Remote Sensing*, **18**, pp. 2869–2875.

Peterson, D.L., Aber, J.D., Matson, P.A., Card, D.H., Swanberg, N., Wessman, C. and Spanner, M., 1988, Remote sensing of forest canopy and leaf biochemical contents. *Remote Sensing of Environment*, **24**, pp. 85–108.

Sala, O.E., Deregibus, V.A., Schlichter, T. and Alippe, H.P.S., 1981, Productivity dynamics of native temperate grassland in Argentina. *Journal of Range Management*, **34**, pp. 48–51.

Schmidt, K.S. and. Skidmore, A.K., 2001, Exploring spectral discrimination of grass species in African rangelands. *International Journal of Remote Sensing*, **22**, pp. 3421–3434.

Schmidt, K.S. and Skidmore, A.K., 2003, Spectral discrimination of vegetation types in a coastal wetland. *Remote Sensing of Environment*, **85**, pp. 92–108.

Sims, D.A. and Gamon, J.A., 2002, Relationship between leaf pigment content and spectral reflectance across a wide range of species, leaf structures and developmental stages. *Remote Sensing of Environment*, **81**, pp. 337–354.

Singh, R.P. and Sirohi, A., 1994, Spectral reflectance properties of different types of soil surfaces. *ISPRS Journal of Photogrammetry and Remote Sensing*, **49**, pp. 34–40.

Soriano, A., Leon, R.J.C., Sala, O.E., Lavado, R.S., Deregibus, V.A., Cahuepé, M.A., Scaglia, O.A., Velázquez, C.A. and Lemmcoff, J.H., 1992, Rio de la Plata Grasslands. In *Ecosystems of the World 8A. Natural Grasslands. Introduction to Western Hemisphere*, R. Coupland (Ed.), pp. 367–408 (Amsterdam: Elsevier).

Taiz, L. and Zeiger, E., 1999, *Plant Physiology* (Sunderland: Sinauer Associates).

THENKABAIL, P.S., SMITH, R.B. and DE PAW, E., 2000, Hyperspectral vegetation indices and their relationships with agricultural crop characteristics. *Remote Sensing of Environment*, **71**, pp. 158–182.

VERHOEF, W., 1984, Light scattering by leaf layers with applications to canopy reflectance modeling: the SAIL model. *Remote Sensing of Environment*, **16**, pp. 125–141.

WAND, S.J.E., MIDGLEY, G., JONES, M.H. and CURTIS, P.S., 1999, Responses of wild C_4 and C_3 grass (*Poaceae*) species to elevated atmospheric CO_2 concentration: a meta-analytic test of current theories and perceptions. *Global Change Biology*, **5**, pp. 723–741.

WESSMAN, C.A., BATESON, C.A. and BENNING, T.L., 1997, Detecting fire and grazing patterns in tallgrass prairie using spectral mixture analysis. *Ecological Applications*, **7**, pp. 493–511.

WRIGHT, I.J., REICH, P.B., WESTOBY, M., ACKERLY, D.D., BARUCH, Z., BONGERS, F., CAVENDER-BARES, J., CHAPIN, T., CORNELISSEN, J.H.C., DIEMER, M., FLEXAS, J., GARNIER, E., GROOM, P.K., GULIAS, J., HIKOSAKA, K., LAMONT, B.B., LEE, T., LEE, W., LUSK, C., MIDGLEY, J.J., NAVAS, M.L., NIINEMETS, U., OLEKSYN, J., OSADA, N., POORTER, H., POOT, P., PRIOR, L., PYANKOV, V.I., ROUMET, C., TJOELKER, S.C.T.M.G., VENEKLAAS, E.J. and VILLAR, R., 2004, The worldwide leaf economics spectrum. *Nature*, **428**, pp. 821–827.