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Article

Estimating Pasture Quality of Fresh Vegetation Based on Spectral Slope of Mixed Data of Dry and Fresh Vegetation—Method Development

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Abstract: The main objective of the present study was to apply a slope-based spectral method to both dry and fresh pasture vegetation. Differences in eight spectral ranges were identified across the near infrared-shortwave infrared (NIR-SWIR) that were indicative of changes in chemical properties. Slopes across these ranges were calculated and a partial least squares (PLS) analytical model was constructed for the slopes *vs.* crude protein (CP) and neutral detergent fiber (NDF) contents. Different datasets with different numbers of fresh/dry samples were constructed to predict CP and NDF contents. When using a mixed-sample dataset with dry-to-fresh ratios of 85%:15% and 75%:25%, the correlations of CP (R^2 = 0.95, in both) and NDF (R^2 = 0.84 and 0.82, respectively) were almost as high as when using only dry samples (0.97 and 0.85, respectively). Furthermore, satisfactory correlations were obtained with a dry-to-fresh ratio of 50%:50% for CP (R^2 = 0.92). The

results of our study are especially encouraging because CP and NDF contents could be predicted even though some of the selected spectral regions were directly affected by atmospheric water vapor or water in the plants.

Keywords: reflectance spectroscopy; spectral slope; pasture quality; crude protein (CP); neutral detergent fiber (NDF); fresh vegetation

1. Introduction

The quality of the plants consumed by livestock in pastures is an important factor for their productivity. The food's potential quality is assessed by indicators such as crude protein (CP) concentration, cell-wall components (NDF—neutral detergent fiber and ADF—acid detergent fiber) and digestibility. The most widely accepted method for assessing these indicators is chemical analysis [1,2] which, although accurate, is both time-consuming and expensive.

Reflectance spectroscopy of solid particles in the visible-near infrared-shortwave infrared (VIS-NIR-SWIR) spectral range is a well-known technique for the rapid and quantitative assessment of chemical composition in many materials [3–5]. This is a rapid, cost-effective, nonchemical and nondestructive technique and for the most part, no sample preparation is needed. In general, vegetation spectra absorb in the VIS range (350–780 nm) due to photosynthetic pigments (centered near 490 and 680 nm), whereas absorption in the NIR (780–1100 nm) and SWIR (1100–2500 nm) domains is associated with water, protein, oil, lignin, starch, sugar, nitrogen and cellulose. For example, protein-associated N-H and C-H bonds absorb at 1510, 1980, 2060, 2130, 2180, 2300 nm and at 1690, 2240, 2350 nm, respectively [6–9].

The synergistic use of VIS-NIR-SWIR reflectance with multivariate statistical methods (such as partial least squares (PLS) regression and principle component analysis) is very useful for extracting quantitative information on the composition and properties of materials such as agricultural products, soils, dust, polymers and drugs (e.g., [10–16]). Remote sensing of foliar biochemicals was developed in the late 1970s [17–20], mainly using methods from laboratory-based NIR spectroscopy (NIRS) [21,22]. Today, NIRS is widely used in the laboratory to identify the chemical composition of plants [9,23–29].

One of the potential applications of NIRS is the analysis of fresh plant material (e.g., leaves, whole plants) without the need for drying or grinding [30]. However, the water content of vegetation poses a major challenge to extending NIRS techniques from the laboratory scale where a dried leaf is under analysis, to analyses in the field or canopy. Indeed, the advantage of conducting spectral measurements on dried, ground vegetation samples lies in the fact that water has a broad absorption range centered around 1400 and 1940 nm that masks other absorption features associated with constituents such as nitrogen, lignin, sugar and cellulose [6]. Cozzolino [30] presents and discusses some of the most recent applications of NIR spectroscopy without the need for drying or grinding. He reports the successful prediction of components such as dry matter, nitrogen, oil and protein in fresh samples, as well as promising results for internal and external quality assessment of mandarin using a portable NIR spectrometer and in-field detection of plant diseases. Predictions of protein content in fresh alfalfa using NIRS have been reported by Petisco *et al.* [31] ($r^2 = 0.68$), Cozzolino and Labandera [32] ($r^2 = 0.86$), and

several others [33,34]. Protein has also been detected in other types of vegetation [35–37]. However, all of these studies made use of the spectrometer's entire spectral range and models were calibrated using fresh vegetation datasets.

In a previous study [28], we found that when using slopes across selected spectral ranges of dried and ground vegetation, it was possible to evaluate several pasture quality indicators with high accuracy, such as CP, NDF and metabolic energy concentration. However, that study was restricted to estimates based only on the spectra of dry samples; adjusting the method to fresh vegetation would enable further *in-situ* analyses, and this was the aim of the present study. Our main objective was to extend the spectral slope method to both dry and fresh vegetation. Importantly, the *main criterion* for spectral slope selection across the entire spectral range was similar spectral behavior between fresh and dry samples, thus avoiding the impact of water absorbance and reducing the amount of data collected by the hyperspectral instrument.

2. Materials and Methods

2.1. Study Area

The study area was Patish basin (31°22'N, 34°40'E), a semiarid region located in the northern Negev Desert of Israel spanning an area of 230 km^2. One of its main land uses is as a natural pasture; others include grazing on stubble wheat fields after harvesting, bare loess soil plains and planted forest. The climate is mostly Mediterranean, with rainfall from November to April averaging 200–300 mm per year [38]. Average daily minimum winter temperatures are 6 °C–8 °C and average daily maximum summer temperatures are 32 °C–34 °C [39]. The area is hilly, with an average height of 200 m above sea level. The soil on the slopes is 1-m deep loess with a sandy loam texture, consisting of 14% clay, 27% silt and 59% sand (USA classification: Calcixerollic, Xerochrepts) on Eocene bedrock [40].

In these shrublands, the gentle slopes are characterized by a continuous matrix of flat soil surface covered with a biological soil crust consisting of bacteria, cyanobacteria, algae, moss and lichen [41]. This crusted intershrub matrix is interspersed with patches associated with shrubs and other large perennials. The natural vegetation includes woody shrubs (<1 m high) and annuals. The dominant perennial species in the research area are the shrubs *Atractylis serratuloides* (Asteraceae), *Noaea mucronata* (Chenopodiaceae) and *Thymelaea hirsuta* (Thymelaeaceae), the geophyte *Asphodelus ramosus* (Liliaceae), and *Tamarix negevensis*. The main annual species are *Reboudia pinnata* (Brassicaceae), *Avena sativa* (Poaceae), *Stipa capensis* (Poaceae), *Hordeum glaucum* (Poaceae), *Bromus scoparius, Crepis sancta, Chrysanthemum coronarium, Scolymus maculatus, Senecio flavus, Atriplex,* and *Centaurea iberica* [42]. The Patish basin site is moderately grazed by Bedouin-owned herds of Awassi sheep. The grazing season is from mid-February to mid-May, when the annuals are at their peak of growth; it ends in mid-spring (March–May), when they are subjected to dry conditions [43].

2.2. Data Collection and Spectral Measurements

The dataset used in this study included fresh and dry pasture samples. The fresh samples were collected at three sites. Two sites were natural pasture, at Gilat Research Center (31°20'36.43"N,

34°40'20.04"E) and Shaked Park (31°16'12"N, 34°39'2.75"E), both long-term ecological research (LTER) sites in the study area; 36 fresh samples were collected on 12 March 2013, after the peak of the rainy season and before the flocks went out to pasture. A third site was selected along the herds' grazing path and 16 samples of fresh and dry vegetation were collected before the rainy season, on 5 November 2012. The entire plant is eaten by the livestock, and therefore the samples included all plant parts-grains, leaves and stems. The samples collected into paper bags to keep them fresh until the end of the collection day, when spectral measurements were taken. The reflectance spectra of the fresh samples were measured in the laboratory using an Analytical Spectral Devices (ASD; Boulder, CO, USA) Fieldspec-Pro JR Spectroradiometer furnished with a contact probe. The ASD measures spectra in 2151 bands at 1-nm intervals across the VIS-NIR-SWIR (350–1000–2500 nm) region. The spectra were measured by direct contact of the probe to the different parts of the vegetation sample, *i.e.*, leaves, stems and grains (where present). The spectra are presented against a white Halon reflectance panel reference (Spectralon, Labsphere Inc.). Each spectral measurement represented an average of 40 spectral readings and the spectra of three replicates for each vegetation sample were averaged. The three replicate measurements were taken from different parts of the plant. Then the vegetation samples were oven-dried for 72 h at 60 °C, ground to pass through a 1-mm sieve, and subjected to chemical analysis for CP and NDF.

The dry sample data consisted of 235 vegetation samples from the pasture area that were obtained from the Agricultural Research Organization (ARO) archives at the Volcani Center, Israel (courtesy of Serge Yan Landau [26,44,45]). The samples had been collected during the grazing season in the years 2002–2011 from the natural pasture area in Patish basin and from sown pasture in experimental farms (Migda and Karei-Deshe', located in the northern Negev and eastern Galilee, respectively) in Israel. The samples were collected from the vegetative bulk of each species, oven-dried for 72 h at 60 °C and ground to pass through a 1-mm sieve [26]. Then spectral measurements and chemical analysis of CP and NDF were performed. The samples were scanned using a Foss NIRS system model 5000 NIR reflectance monochromator spectrometer (Hoganas, Sweden) at 1104–2492 nm in 2-nm increments (700 bands), set to collect NIR spectra as log (1/R) where R is the reflectance [44]. Each spectral measurement represented an average of 25 spectral readings. The spectra of two replicates for each vegetation sample were averaged.

2.3. Chemical Reference

We used % CP and % NDF as indicators of pasture quality. The chemical analysis for CP was performed by automated Kjeldahl method and for NDF according to Goering and Van Soest [1,27]. Protein content was determined by acid digestion [46]. Neutral detergent fiber (NDF) was measured using a detergent that solubilizes the proteins and sodium sulfite and helps remove some of the nitrogenous matter; EDTA was used to chelate calcium and remove pectin at boiling temperatures; triethylene glycol was used to remove non-fibrous matter from concentrate feeds, and heat-stable amylase was used to remove starch. Amylase was added twice (once during reflux and once during filtration) to minimize filtering difficulties. Heat-stable amylases were used in hot solutions to inactivate potential contaminating enzymes that might degrade fibrous constituents. NDF content was determined as {((crucible weight + fiber) − crucible weight w/o fiber)/(sample weight × lab dm as decimal)} × 100 [47].

2.4. Slope Calculation and Data Analyses

To simplify the spectral signals, thereby ensuring stable calibration, and to improve the predictive ability of the final model, we applied different pretreatments to the spectral domain. The spectral data were considered in reflectance and absorbance ($-\log_{10}R$). In addition, continuum removal (CR) spectra were calculated. The commonly used CR technique [48–51] normalizes reflectance spectra and enhances spectral differences, enabling the distinction and highlighting of individual absorption features from a baseline. In this algorithm, the monotonous spectra are characterized by a reflectance signal value of one, and absorption features are presented relative to a continuum of interpolated reflectance values that connect the two absorption edges. Then, slopes are calculated with the following equation:

$$m = \frac{y_2 - y_1}{x_2 - x_1} \quad (1)$$

where the x axis represents wavelength and the y axis represents CR reflectance.

To develop a model to assess pasture quality, we first used CR spectra to visually inspect the spectral behavior *vs.* chemical information on the vegetation. Then, the differences in spectral behavior as a function of wavelength, which are indicative of changes in chemical properties, were identified, yielding eight spectral ranges (Table 1). The slopes across these spectral ranges were then calculated. Importantly, the *main criterion* for spectral slope selection across the entire spectral range was similar spectral behavior between the fresh and dry samples.

2.5. Data Processing and Quantitative Analyses

The ASD spectral data (1501 bands between 1000 and 2500 nm) of the fresh vegetation were resampled to match the Foss NIRS system model 5000 (700 bands between 1104 and 2492 nm) and the slopes were then calculated. PLS regression analysis was used with the sloped base model to predict the CP and NDF values of fresh vegetation.

Selection of a suitable calibration set is of critical importance in any PLS analysis. The overall goal is to meet a number of requirements: (i) it must be representative of the future population from which the new X (spectral) measurements will be sampled; (ii) it should cover all possible variations in the measurement conditions that might impact a multivariate calibration; (iii) measuring conditions should be as similar as possible; (iv) it must span the X (spectral) space, as well as the Y (reference) dynamic space as widely and representatively as possible [52].

We used the most popular "full cross-validation" method where only one sample at a time is kept out of the calibration and used for prediction (also termed the "leave one sample out procedure"). The dataset was split into two independent subsets: a calibration set using different subdivisions of samples, and an external test set to assess the accuracy of the constructed model. In both, dry and fresh vegetation was combined into one dataset. Since there were two populations and slopes that generally described both subsets (dry and fresh), the impact of different numbers of samples in the calibration set on the prediction of CP and NDF was investigated. For the calibration dataset, the following subdivisions were tested: 85 *vs.* 15, 75 *vs.* 25, 50 *vs.* 50, 35 *vs.* 65 (dry *vs.* fresh sample, respectively). Each PLS model was run and tested on different numbers of dry and fresh samples based on the main

described criterion. An external test set comprised of 5 to 12 fresh samples and 20 to 35 dry samples was used to examine the model's predictive ability.

2.6. PLS Data Analyses

The PLS regression is generally based on latent variable (LV) decomposition of two blocks of variables, the X and Y matrices, which contain spectral data and any reference chemical variable, respectively. The objective of the regression is to locate small numbers of PLS components that efficiently predict Y when X is used [52]. PLS regression has advantages over other regression techniques, such as stepwise multiple linear regression and principal component regression. It not only works with multicollinear variables, but also when the number of independent variables is greater than the number of observations, which is generally the case for NIRS analyses [53].

The ultimate goal of multivariate regression analysis is to create a calibration equation (or series of equations) which, when applied to data of "unknown" samples measured in the same manner, will accurately predict the quantities of the constituents of interest [52]. The multivariate calibration models were generated using PLS regression, with the goal of defining a relationship between the eight spectral slopes in the NIR-SWIR spectral range of pasture samples and each chemical reference (*i.e.*, CP and NDF):

$$Y=A + A_1X_1 + A_2X_2 + A_3X_3 + \ldots + A_nX_n \quad (2)$$

where Y is the chemical reference of a sample, A is an empirical coefficient, and X_i is the spectral slope in the i^{th} wavelength region.

Statistical parameters for the calibration model were calculated by leave-one-out cross validation (only one sample at a time is kept out of the calibration and used for prediction). The performance and relevance of the PLS regression models were further evaluated by computing different statistics. The difference between the predicted values and measured chemical reference values was expressed as the root mean square error of prediction (RMSEP) or the root mean square error of cross validation (RMSECV). RMSEP is defined as the square root of the average of the squared differences between the predicted and measured values of the validation objects [52]:

$$RMSEP = \sqrt{\frac{\sum (X_m - X_p)^2}{n_y}} \quad (3)$$

where X_m is the chemically measured value of a sample, X_p is the predicted value of the sample based on the spectral analysis, and n_v is the number of samples in the calibration stage.

In addition, we used the ratio of prediction to deviation (RPD), which is defined as the ratio of the standard deviation of the reference values (e.g., protein) to the RMSECV or RMSEP. An RPD value below 1.5 was taken to indicate that the model is unusable, a value between 1.5 and 2.0 that it has the potential to distinguish between high and low values, and between 2.0 and 2.5, quantitative prediction is possible. RPD values above 2.5 were considered to indicate excellent predictive capability of the model [54].

2.7. Calculating the Water-Absorption Area

One of the most spectrally pronounced factors, in addition to leaf structure, foliar pigments, lignocellulose absorbance, *etc.*, is the total amount of water present in the leaf, which affects the

degree to which incident solar energy in the NIR-SWIR region is absorbed by vegetation [55]. In this regard, calculating the area under the curve (mathematically known as the definite integral) can be a good indicator of water concentration in vegetation. First, two maximum points of the absorption shoulders (x1 , y1 and x2 , y2; Figure 1) were identified. Then, the coefficients of the straight-line equation that is tangent at these points were calculated:

$$a = (y2 - y1)/(x2 - x1),\ b = y1 - ax \quad (4)$$

Finally, the area between the straight line and the absorption line was calculated (Figure 1) by:

$$\text{Total area} = \sum_{i=x1}^{x2} \left(y_{L(i)} - y_{C(i)}\right) \quad (5)$$

where *x1 − x2* is the spectral range of the absorption water, y_L is the CR value at *x(i)* of the straight line and y_C is the CR value at *x(i)* of the absorption line.

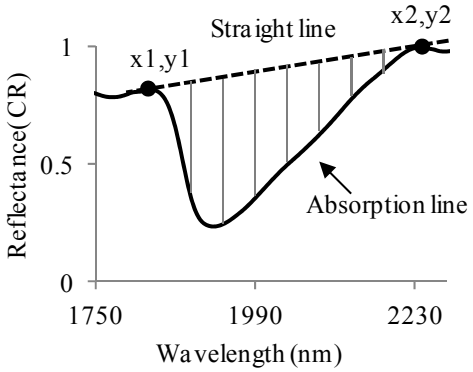

Figure 1. Demonstration of absorption area calculation.

3. Results and Discussion

3.1. Chemical Reference: CP, NDF

Figure 2 shows the distribution of CP and NDF in dry and fresh vegetation. The range of CP values in the dry vegetation was wider than that in the fresh vegetation, whereas the range of NDF values was similar. The range of CP and NDF values in the fresh vegetation was 3.4%–17.2% (48 samples, average 9.5% and STD (standard deviation) 3.9%), and 27%–71% (43 samples, average 51.5% and STD 11.8%), respectively. The range of CP and NDF values of the dry vegetation was: 2.5%–32% (224 samples, average 9.46% and STD 6.9%), and 28%–76% (232 samples, average 59.4% and STD 10.9%), respectively.

3.2. Spectral Slope Analysis

Figure 3 presents the reflectance (a) and continuum removal (CR) (b) spectra of three fresh vegetation samples and three dry vegetation samples with different percentages of CP. The reflectance measurements were performed with two spectroradiometers: the Foss NIRS system for the dry samples and the ASD Fieldspec-Pro JR for the fresh samples. The baseline of the spectral measurements differed between the two spectroradiometers (Figure 3a) due to differences in their illumination intensity, physical state of the samples (powdered for dry *vs.* fresh) and vegetation water content. In

contrast, when presented in CR (Figure 3b), the differences between the CR spectral slopes were more pronounced than those of the reflectance spectra. On the one hand, the CR spectra emphasize the absorption features of the chemical chromophore and on the other, they reduce the physical chromophore effect [56].

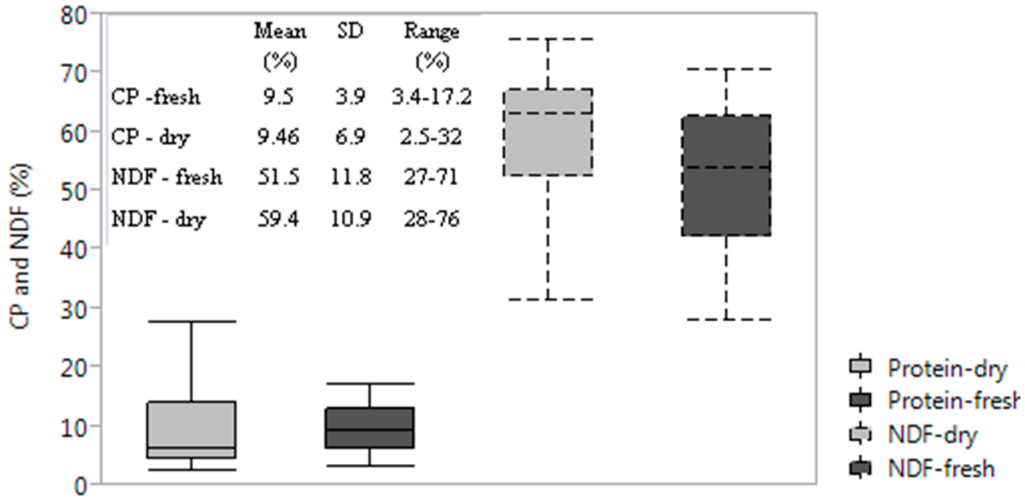

Figure 2. Distribution of crude protein (%CP) and neutral detergent fiber (%NDF) contents in dry and fresh vegetation.

Finding a similar change in spectral slope for both dry and fresh vegetation would enable overcoming the problem of strong absorbance features that mask chemical chromophores. In Figure 3b , c, we show the selection of spectral slopes for dry and fresh vegetation. Changes in slope as a function of different CP and NDF contents could be seen in eight spectral ranges: 1748–1764 nm, 1766–1794 nm, 2070–2088 nm, 2278–2286 nm, 2334–2344 nm, 1940–2226 nm, 2024–2090 nm and 2090–2160 nm.

In general, the vegetation spectra at 1440 and 1940 nm exhibit a broad and strong water-absorption feature that reduces reflectance [55]. This reduction is especially pronounced for fresh vegetation. In dry vegetation, the water absorption no longer conceals the absorption features, such as those at 1773 nm, and 2330 nm which are caused by organic bonds of plant biochemicals due to the presence of proteins, lignin, and cellulose [49]. A detailed description of the main absorbance features of the vegetation can be found in Curran [6] and Schwanninger *et al.* [9]. Note that CR spectra, in contrast to reflectance spectra, of both fresh and dry vegetation (Figure 3a,b) exhibit weaker absorption features at ~2330 nm primarily due to the presence of nitrogen-containing compounds in plants [49]. Furthermore, in Figure 3c we show an example of this relationship by zooming in on the 2334–2344 nm spectral range, where the slope of both fresh and dry vegetation is seen to decrease with increasing CP content. In other words, despite the relatively strong absorption features of water, the CR spectra in both dry and fresh vegetation generally exhibit similar spectral behavior, even though the water-absorption features are much stronger in the latter.

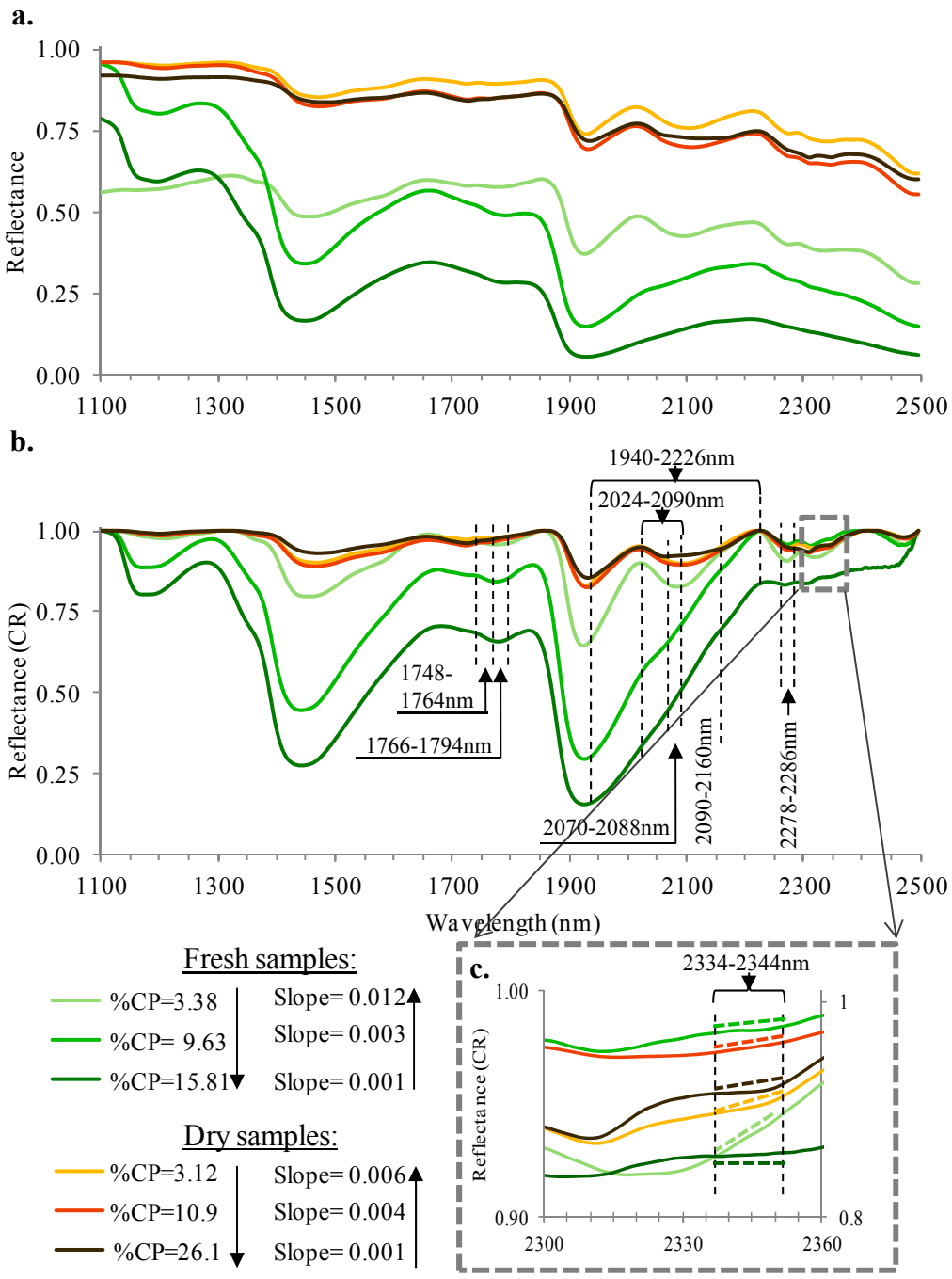

Figure 3. (**a**) Reflectance and (**b**) continuum removal (CR) spectra of fresh and dry vegetation samples with different percentages of crude protein (CP). (**c**) Zooming in on the 2334–2344 nm spectral range to demonstrate the changes in slope with changes in CP concentration. Note the variability in the slopes across the different percentages of CP in the dry and fresh samples. Specifically, as the slope decreases, the CP value increases.

The distribution of the slopes for the eight spectral ranges is presented in Figure 4. In the upper panel, variability is seen in the spectral slopes at 1748–1764 nm, 1766–1794 nm, 2278–2286 nm and 2334–2344 nm, presumably due to differences in chemical composition and chromophores, which differ for each spectral range. The slopes of the dry samples are similar to those of the fresh ones, except for the slope calculated for the 1748–1764 nm spectral range. The bottom of Figure 4 shows

significant variability in the spectral slopes at 2070–2088 nm, 1940–2226 nm, 2024–2090 nm and 2090–2160 nm due to water content. The spectral slope calculated on the long wavelength side of water absorbance at 1940 nm (Figure 3b) can explain the high variability and differences in slope values between dry and fresh samples (Figure 3b). Specifically, the strong water absorbance at 1940 nm leads to a high slope value for fresh *vs.* dry vegetation (Figure 3c). Note that the range of slope values in the spectral ranges 1766–1794, 2278–2286, 2334–2344 is similar for fresh and dry vegetation. This presumably allows modeling changes related to the presence of CP and NDF, despite the presence of water in the fresh samples.

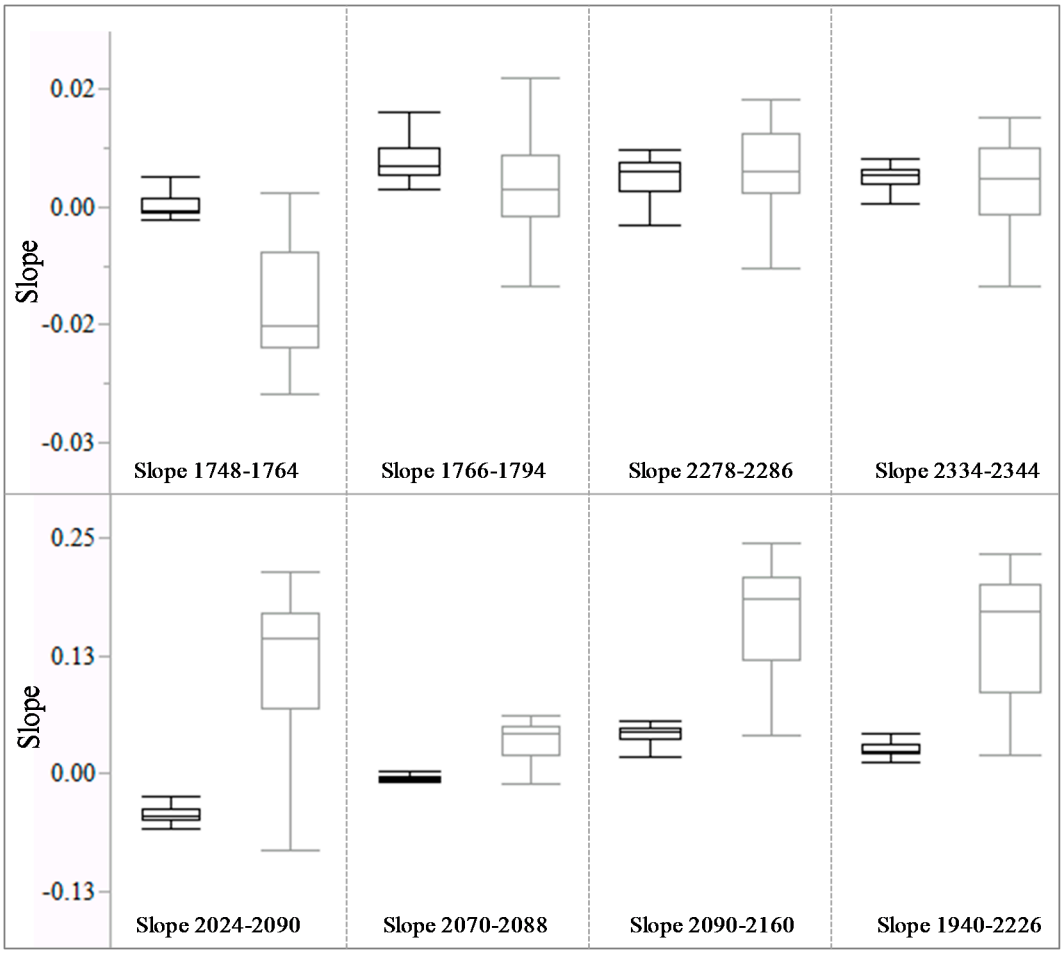

Figure 4. Slope value distribution of dry (black) and fresh (gray) vegetation for 8 spectral ranges.

3.3. PLS Analysis

Table 1 summarizes the correlation between every specific slope range and the CP and NDF contents of fresh vegetation using simple linear regression analyses. Although the correlations were not high (for CP: 0.14–0.42 and for NDF: 0.15–0.49), they suggest the possibility of using the slopes of several ranges to assess chemical constituents. When all dry and fresh samples were used to construct a PLS model, the use of eight slopes as independent variables gave a best-fit model for the CP assessment of the fresh samples, and using 7 out of 8 slope ranges (excluding 1940–2226 nm) gave a best-fit model for NDF (Table 2, see 85%:15% ratio). For CP, the R^2 was 0.96, 0.95 and 0.95, RMSE

was 2.34, 2.52 and 1.45 and RPD was 2.50, 2.70 and 4.80 (calibration, validation and prediction on the external test set data, respectively). For NDF, the R^2 was 0.98, 0.98 and 0.84, RMSE was 7.49, 7.57 and 4.75 and RPD was 1.68, 1.67 and 2.59 (calibration, validation and prediction on the external test set data, respectively). Figures 5 and 6 present the distribution of the respective predicted CP and NDF values versus reference values of the external test set samples. The accuracy of the model prediction was relatively high.

Table 1. Correlation between slopes in selected spectral regions and crude protein (CP) and neutral detergent fiber (NDF) contents of fresh vegetation.

	CP	NDF
Slope Spectral Range (nm)	**R^2 (n = 48)**	**R^2 (n = 43)**
1748–1764	0.2804	0.1943
1766–1794	0.193	0.2258
2070–2088	0.4225	0.276
2278–2286	0.2415	0.3043
2334–2344	0.4149	0.4889
2090–2160	0.2387	0.15
2024–2090	0.3877	0.2422
1940–2226	0.1435	0.0884

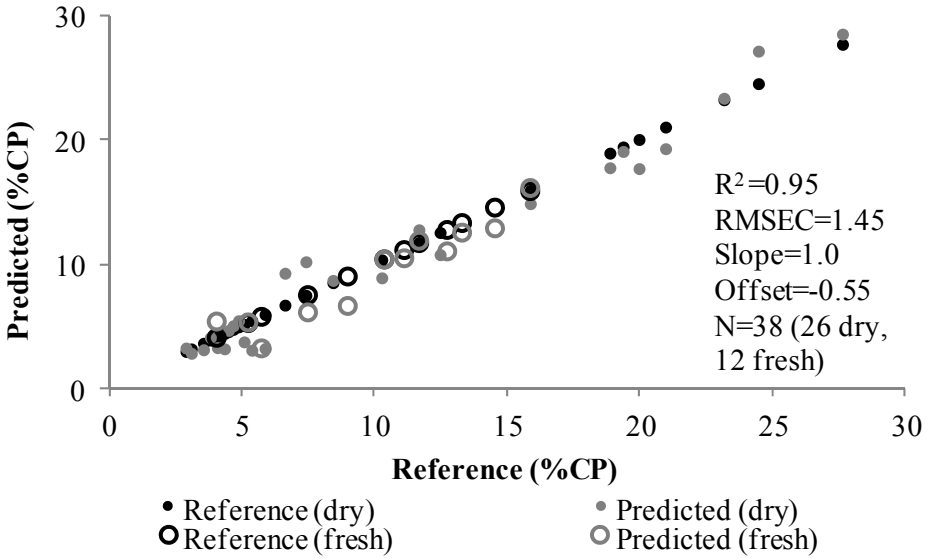

Figure 5. Crude protein (CP) distribution from predicted (external test data) *vs.* reference values of fresh and dry vegetation using partial least squares (PLS) model.

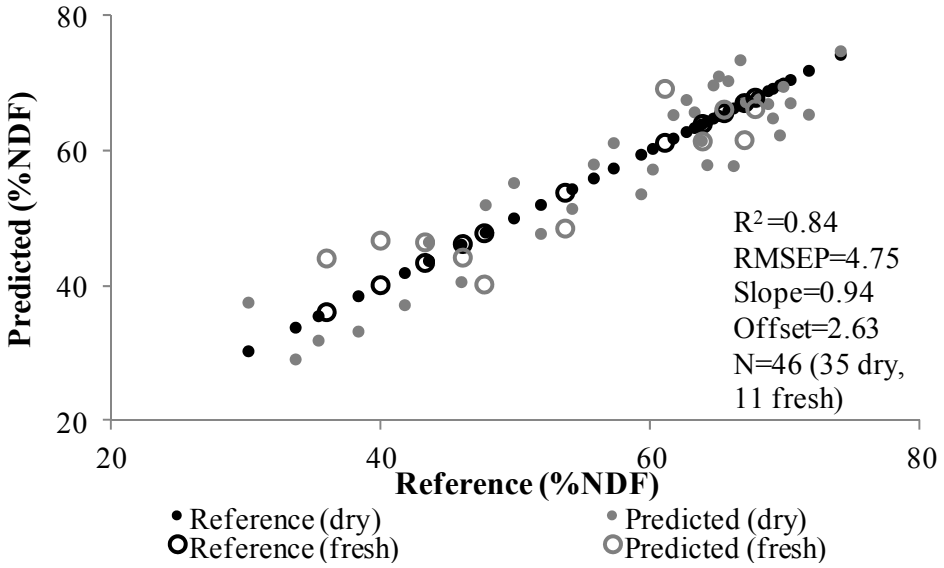

Figure 6. Neutral detergent fiber (NDF) distribution from predicted (external test data) *vs*. reference values of fresh and dry vegetation using partial least squares (PLS) model.

A much clearer picture of spectroscopic pasture-quality changes compared to the original reflectance (Figure 3), especially their importance for the calibration, was obtained from a score plot of samples from the PLS model for CP (Figure 7). To assess CP and NDF, the first three and two, respectively, LV components in the PLS model explained 100% of the X variance (slope spectra), and 83% (for CP) and 98% (for NDF) of the Y variance (chemical components). This indicated that most of the spectral variation in the eight selected spectral domains is related to the CP, NDF and water components which were modeled by PLS. Furthermore, a score plot of samples from PLS modeling demonstrated the good correlation between slope spectra and chemical constituents (CP and NDF) with an increase from the top down. The score plot for the CP model (Figure 7) indicated that a significant part of the spectral variations observed in the dry and fresh samples are indeed related to the protein, as predicted by the PLS model. There was an increasing trend in CP content among samples from the top down (Figure 7) following a slight diagonal, presumably due to the difference in water content. To further investigate this assumption, we calculated the total absorption area between 1838 and 2238 nm (the spectral range between the shoulders of the absorption peak of water at 1940 nm), used as an indicator of water content in materials.

Figure 8 presents the absorption area of two dry samples with similar CP content (see Figure 7a) and two fresh samples with similar CP content (see Figure 7c). The difference in water content between the dry and fresh samples is remarkable (Figure 8, right), as are the differences in water content between the two dry samples and between the two fresh samples (Figure 8, left). The calculated results of the total area of water absorption were uploaded into the score plot and are presented in Figure 9. When moving from left to right on the plot, there is an increase in water content among samples. The top down and slightly diagonal tendency of changing CP content and right to left change in the water content occurred in all three groups of vegetation: dry, partially dry and fresh (Figures 7 and 9). The partially dry samples were collected in November, before the rainy season, at Nachal Patish basin, and are therefore located between the dry and fresh samples on the plot. In this context, Figures 7 and 9 reflect the fact that,

despite the original reflectance containing high water absorption, the LV components are direct approximations of the pure chemical (CP, NDF, water) [57,58], influencing the calibration. Importantly, for fresh vegetation, slopes in the spectral ranges 2070–2088 nm and 2334–2344 nm (Table 1) are relatively sensitive to increasing/decreasing CP/NDF amounts relative to the rest of the calculated slopes.

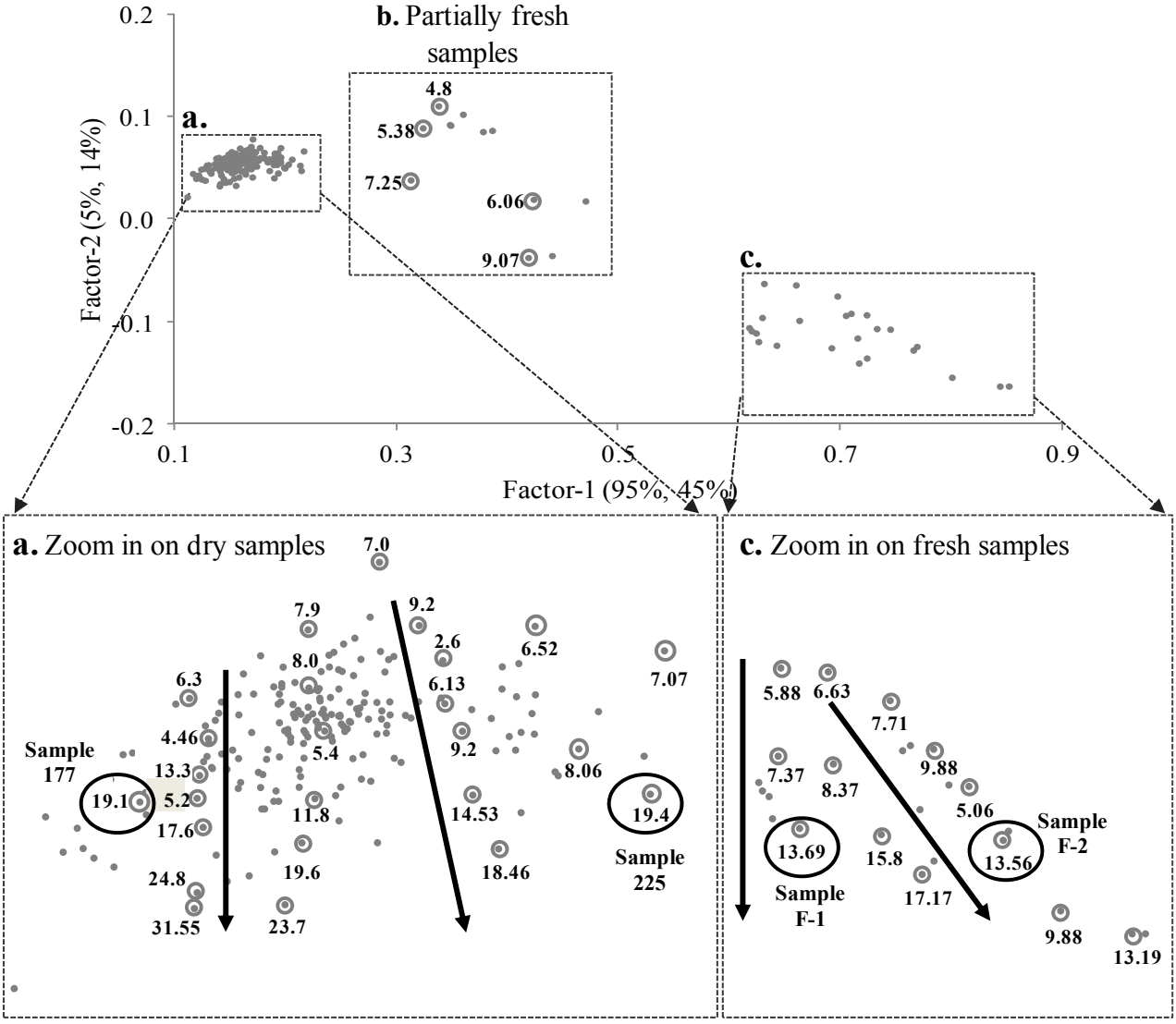

Figure 7. Score plot for the partial least squares (PLS) model for crude protein (CP) using eight slopes. Numbers next to gray circles represent the protein values (reference). Black circles indicate two specific dry samples (177 and 225) and two specific fresh samples (F-1, F-2). Arrows indicate the change from top to bottom in protein content measured in the samples. (**a**) Zoom in on dry samples, (**b**) Partially fresh samples, (**c**) Zoom in on fresh samples.

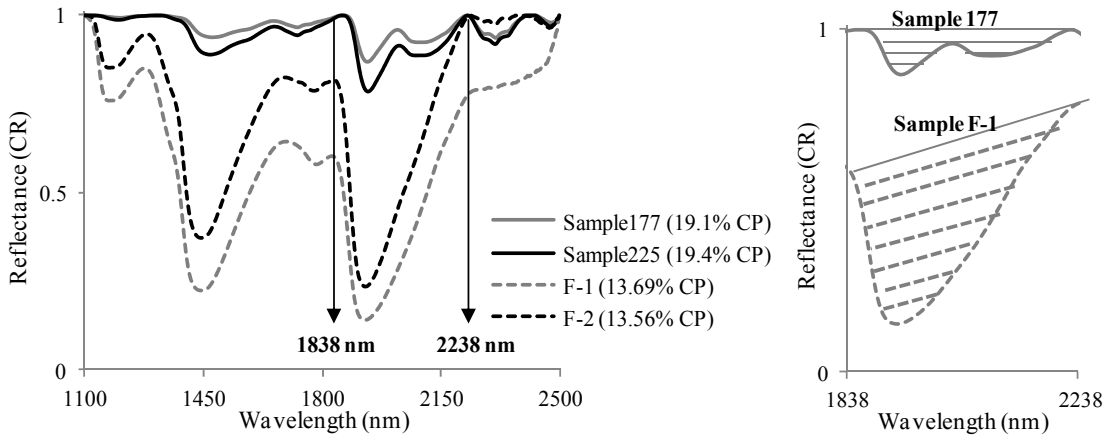

Figure 8. Continuum removal (CR) of four samples to demonstrate the difference in absorption area between the dried and fresh samples.

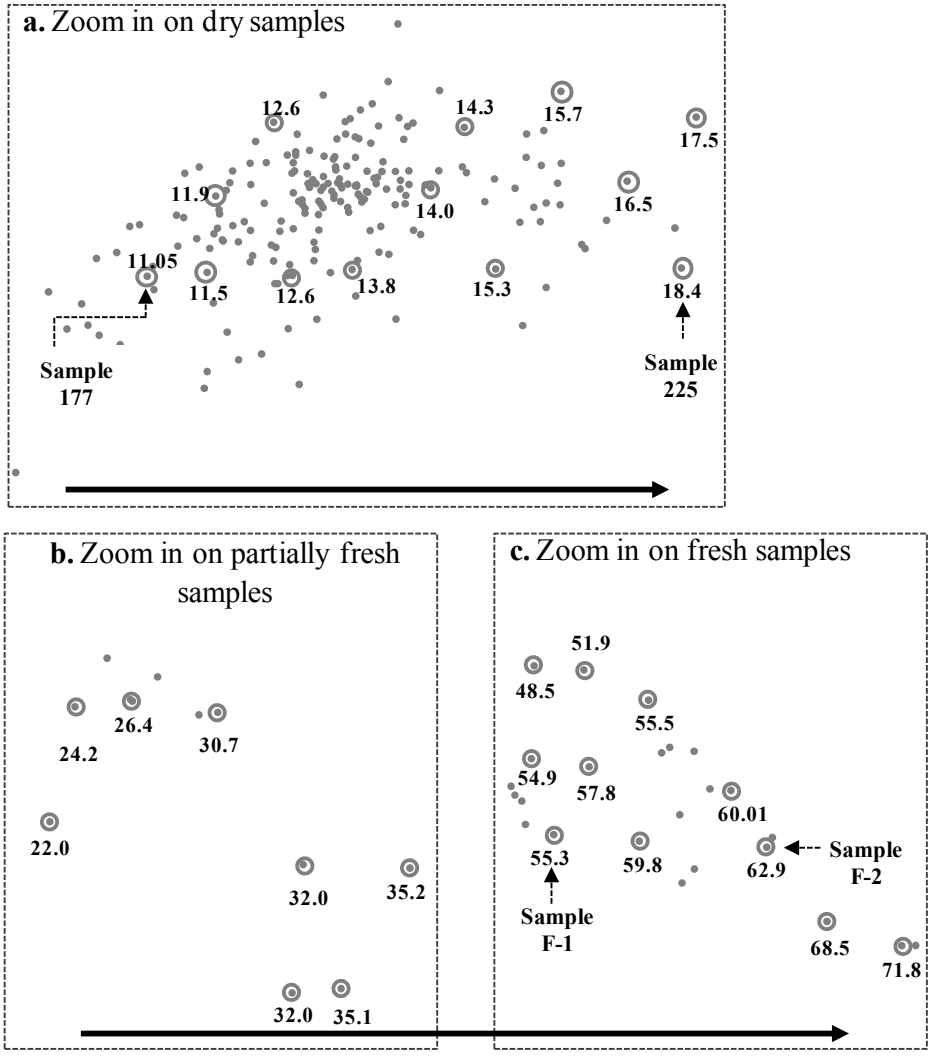

Figure 9. Result of uploading the total area absorption into the score plot of the partial least squares (PLS) model for crude protein (CP). Numbers next to gray circles represent the total area of water absorption. (**a**) Zoom in on dry samples, (**b**) Zoom in on partially fresh samples, (**c**) Zoom in on fresh samples.

Table 2 presents the impact of using different numbers of samples in the calibration and validation sets on the prediction of CP and NDF. When only fresh samples were used to construct the model (Table 2, 100% fresh samples), the accuracy of the CP and NDF predictions was low (*i.e.*, not applicable for CP and R^2 = 0.56 for NDF). On the other hand, when using only dry samples, the accuracy of the estimates for dry samples only was very high (Table 2, 100% dried samples). To identify the required threshold ratio to predict CP and NDF content in fresh vegetation, as mentioned in section 2.5, we reduced the original proportion of the dried samples from 85% to 75%, 50% and 35%, and PLS regression models were constructed (Table 2). In general, as the number of dry samples decreased, the R^2, slope and RPD decreased, whereas the RMSE increased. We selected only the fresh samples from the prediction dataset and ran a simple regression analysis between the predicted and reference values to compare the results of the combined model predictions as might be obtained in future *in-situ* measurements. These results are presented in Figure 10. As the proportion of dry samples increased in the dataset, the total accuracy of the model improved. When we consider both these regression results (Figure 10) and RPD values (Table 2), we conclude that the threshold for satisfactory prediction of CP and NDF content of fresh vegetation requires 50% : 50% and 75% : 25% dry-to-fresh vegetation, respectively.

Table 2. Partial least squares (PLS) regression model results of different ratios of dry to fresh vegetation samples and predicted crude protein (CP) and neutral detergent fiber (NDF) contents.

	CP Model Statistical Characteristics			**NDF Model Statistical Characteristics**		
	Calibration	**Validation**	**Prediction**	**Calibration**	**Validation**	**Prediction**
100% Dry Samples						
Total dry samples	198	198	26	197	197	35
Slope	0.95	0.94	0.99	0.96	0.96	0.98
Offset	0.34	0.36	-0.15	1.56	1.60	0.32
RMSE	1.76	1.82	1.33	6.12	6.17	4.69
RPD	3.74	3.62	5.92	1.96	1.94	2.72
R^2	0.98	0.97	0.97	0.99	0.99	0.85
85%:15% (Dry/Fresh Samples)						
Total dry samples	198	198	26	197	197	35
Total fresh samples	36	36	12	32	32	11
Slope	0.91	0.90	1.01	0.90	0.90	0.95
Offset	0.55	0.64	-0.55	4.98	5.10	2.63
RMSE	2.34	2.52	1.45	7.49	7.57	4.75
RPD	2.50	2.70	4.80	1.68	1.67	2.59
R^2	0.96	0.95	0.95	0.98	0.98	0.84
75%:25% (Dry/Fresh Samples)						
Total dry samples	122	122	23	113	113	32
Total fresh samples	38	38	10	31	31	11
Slope	0.90	0.89	1.01	0.88	0.87	0.95
Offset	0.62	0.75	-0.78	5.78	6.01	2.60
RMSE	2.63	2.82	1.26	8.18	8.32	4.63
RPD	2.43	2.27	5.15	1.59	1.57	2.49
R^2	0.95	0.94	0.95	0.98	0.97	0.82

Table 2. *Cont.*

	CP Model Statistical Characteristics			NDF Model Statistical Characteristics		
	Calibration	**Validation**	**Prediction**	**Calibration**	**Validation**	**Prediction**
50%:50% (Dry/Fresh Samples)						
Total dry samples	42	42	10	43	43	10
Total fresh samples	38	38	10	32	32	10
Slope	0.89	0.86	0.92	0.78	0.77	0.97
Offset	0.64	0.99	0.43	11.2	11.7	3.01
RMSE	2.74	3.19	1.75	8.9	9.2	8.3
RPD	2.18	1.89	3.74	1.42	1.39	1.63
R^2	0.94	0.92	0.92	0.97	0.97	0.51
35%:65% (Dry/Fresh Samples)						
Total dry samples	20	20	5	22	22	5
Total fresh samples	38	38	10	31	31	10
Slope	0.89	0.86	0.73	0.69	0.67	0.81
Offset	0.67	1.05	1.73	15.7	16.6	11.1
RMSE	2.88	3.36	2.14	9.69	9.99	8.43
RPD	2.15	1.87	1.97	1.27	1.23	1.55
R^2	0.94	0.91	0.72	0.96	0.96	0.50
100% Fresh Samples						
Total fresh samples	38	38	10	33	33	10
Slope	0.4	0.38	-0.08	0.47	0.48	0.58
Offset	5.32	5.54	11.7	25.47	25.6	23.9
RMSE	3.18	3.3	4.2	11.5	12.2	8.04
RPD	0.87	0.84	0.35	0.99	1.00	1.20
R^2	0.89	0.88	NA	0.95	0.94	0.56

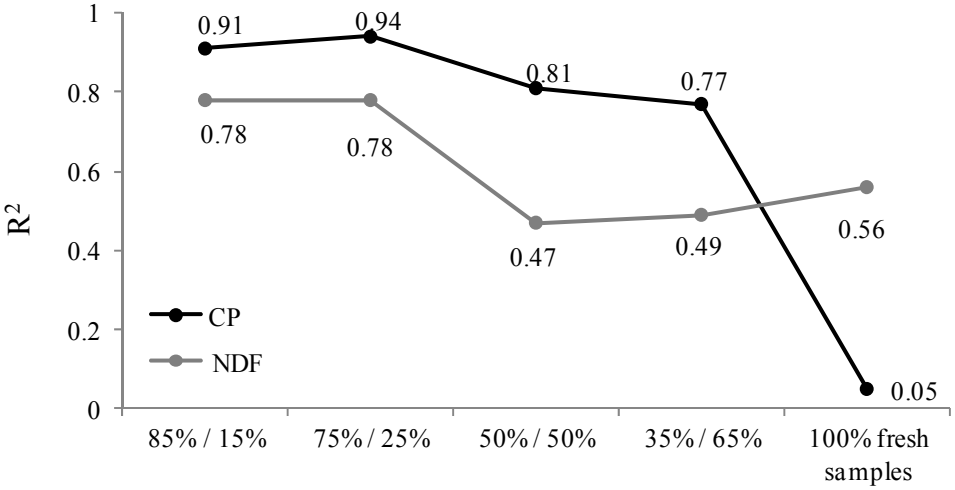

Figure 10. Regression between the reference and prediction for fresh samples only from the external dataset. R^2 decreases with number of dried samples.

A much clearer picture of spectral slope *vs.* changes in CP content is obtained from a plot of regression coefficients *vs.* spectral slope for each PLS model (Figure 11). Three slopes were found to be significant for all models: 2070–2088 nm, 2024–2090 nm and 2090–2160 nm. Note that these three slope ranges are fully within the wide water absorbance around 1940 nm in the CR reflectance

(Figure 3b). This strengthens our assumption that it possible to predict CP and NDF of fresh vegetation using mixed spectral information of dried and fresh vegetation samples.

Importantly, to compare the accuracy of the spectral slope approach, PLS analyses were run on the entire spectral range. To that end, reflectance values were considered as first-derivative values and the PLS model was run using the same calibration samples as the spectral slope models. Although the overall accuracy of the constructed model comprised of 85% dry vegetation and 15% fresh vegetation was excellent (R^2 = 0.95, slope = 0.88, RPD = 2.7), the model prediction for the external validation dataset was not satisfactory for fresh samples (R^2 = 0.85 for the whole external dataset, but R^2 = 0.032 when dry samples were excluded). In contrast, the spectral slope approach generated comparatively high prediction accuracy, albeit less so for fresh samples (using a similar comparison, Figure 10).

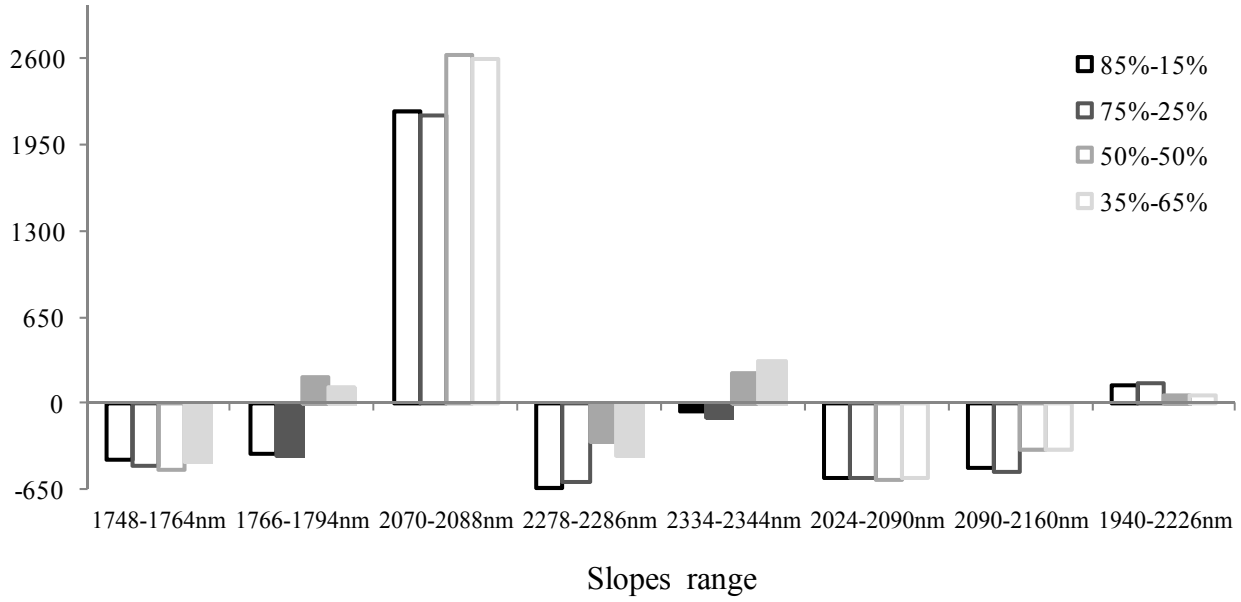

Figure 11. Regression coefficients of partial least squares (PLS) crude protein (CP) model for different ratios of dry to fresh vegetation samples (filled rectangle indicates less important slope).

Many studies have used spectral reflectance data collected both *in situ* at the field level (fresh) and in the laboratory (dry) to measure forage quality, using chemical components such as NDF, ADF, digestible energy, nitrogen and protein. Guo *et al*. [59] used six known chemical absorption regions, and showed that at the field level, a prediction of protein in mixed grass species is possible (R^2 = 0.63). However, the relationship between canopy reflectance and the other forage quality variables was not very strong. Starks *et al*. [60] found that calibration equations could be developed from reflectance data collected from live standing grass canopies to predict nitrogen (R^2 = 0.76), NDF (R^2 = 0.63) and ADF (R^2 = 0.69). Zhao *et al*. [61] compared several different methods of data analysis to predict forage quality using canopy reflectance measurements. Their results indicated the potential of using canopy reflectance data to estimate forage quality variables of warm-season grass pastures (R^2 = 0.27–0.72 for NDF and 0.67–0.74 for CP). Furthermore, Adjorlolo *et al*. [62] found that using a spectral resampling technique for a few strategically selected band centers of known absorption or reflectance features is sufficient to estimate forage nutrients. Their results indicated prediction accuracies for CP content ranging from R^2 = 0.51 to 0.62.

Our study, based on the spectral slope approach, indicated accurate prediction of CP and NDF (R^2 values of 0.92–0.95 and 0.82–0.84, respectively; Table 2). Therefore, we suggest further testing this method for future field studies.

4. Conclusion

In this study, we hypothesized that changes in the spectral slopes of dried/ground and fresh vegetation samples can be used for the quantitative assessment of plant composition of fresh vegetation. Eight spectral regions across the NIR-SWIR region were identified: 1748–1764 nm, 1766–1794 nm, 1940–2226 nm, 2070–2088 nm, 2024–2090 nm 2090–2160 nm, 2278–2286 nm and 2334–2344 nm. Slopes across these ranges were calculated and PLS analytical models were constructed for the slopes *vs*. CP and NDF contents. When using a mixed-sample dataset with a dry-to-fresh vegetation ratio of 85%–15%, the correlation was almost as high as when using only dry samples. Furthermore, we found that when the ratios between dry and fresh samples are 50%:50% and 75%:25%, a satisfactory prediction of CP and NDF content, respectively, in fresh vegetation was obtained.

Importantly, it was also found possible to combine spectral measurements from different spectroradiometers—the Foss NIRS system and ASD Fieldspec-Pro JR (laboratory and field spectrometer, respectively). The ability to use mixed vegetation samples and different spectrometers in PLS modeling is important, because the spectral information acquired using different instruments and chemical references from dried plants are available, and all that remains is to apply the proposed method to fresh vegetation samples.

Finally, the results of our study are especially encouraging because even though some of the selected spectral regions are directly affected by atmospheric water vapor or water in the plants, it is still possible to predict CP and NDF contents. Therefore, the slope method can be further adapted to evaluate the quality of various types of vegetation in *in-situ* analyses of pasture areas using a field spectrometer. To that end, future *in-situ* studies that consider field canopy reflectance, passive light source (sunlight) and mixed pixels of vegetation and soil are warranted.

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Author Contributions

Naftaly Goldshleger conceived the project and a project PI. Rachel Lugassi, Naftaly Goldshleger and Eli Zaady, designed, performed and supported the experiments. Rachel Lugassi analyzed the data.

Rachel Lugassi and Alexandra Chudnovsky performed the statistical analysis, modeling and wrote the paper. Levana Dvash contributed materials. All authors contribute in paper writing.

Conflicts of Interest

The authors declare no conflict of interest.

References

1. Goering, H.K.; Van Soest, P.J. Forage fiber analysis (apparatus, reagents, procedures and some applications). In *Agriculture Handbook No. 379*; Agriculture Research Service, United States Department of Agriculture: Washington DC, USA, 1970; pp. 1–20.
2. AOAC International. *Official Methods of Analysis of AOAC International*, 16th ed.; Association of Official Analytical Chemists: Arlington, VA, USA, 1995.
3. Carey, W.P.; Wangen, E. Determining chemical characteristics of plutonium solutions using visible spectrometry and multivariate chemometric methods. *Chemometrics Intelligent Lab. Syst.* **1991**, *10*, 245–257.
4. Coscione, A.R.; Andrade, J.C.; van Raij, B.; Abreu, M.F. An improved analytical protocol for the routine spectrophotometric determination of exchangeable aluminum in soil extracts. *Commun. Soil Sci. Plant Anal*. **2000**, *31*, 2027–2037.
5. Evrendilek, F.I.C.; Kilic, S. Changes in soil organic carbon and other physical soil properties along adjacent Mediterranean forest, grassland, and cropland ecosystems. *Turkey. J. Arid Environ.* **2004**, *59*, 743–752.
6. Curran, P.J. Remote sensing of foliar chemistry. *Remote Sens. Environ*. **1989**, *30*, 271–278.
7. Murray, I.; Williams, P.C. Chemical principles of near infrared technology. In *Near Infrared Technology in Agriculture and Food Industries*; Wiliams, P.C.; Noriss, K.H., Eds.; American Association of Cereal Chemistry Inc.: St. Paul, MN, USA, 1987; pp. 17–31.
8. Workman, J. Jr.; Weyer, L. *Practical Guide to Interpretive Near-Infrared Spectroscopy*; CRC Press, Taylor & Francis Group: Boca Raton, FL, USA, 2008**.**
9. Schwanninger, M.; Rodrigues, J.C.; Fackler, K. A review of band assignments in near infrared spectra of wood and wood components. *J. Near Infrared Spectroscopy* **2011**, *19*, 287–308.
10. Blanco, M.; Villarroya, I. NIR spectroscopy: a rapid-response analytical tool. *Trends Anal. Chem.* **2002**, *21*, 240–250.
11. Dematte, J.A.M.; Campos, R.C.; Alves, M.C.; Fiorio, P.R.; Nanni, M.R. Visible-NIR reflectance: a new approach on soil evaluation. *Geoderma* **2004**, *12*, 95–112.
12. Ben-Dor, E.; Banin, A. Near-infrared analysis as a method to simultaneously evaluate several soil properties. *Soil Sci. Soc. Am. J.* **1995**, *59*, 364–372.
13. Marten, G.C.; Brink, G.E.; Buxton, D.R.; Halgerson, J.L.; Hornstein, J.S. Near infrared reflectance spectroscopy analysis of forage quality in four legume species. *Crop Sci.* **1984**, *24*, 1179–1182.
14. Waters, C.J.; Givens, D.I. Nitrogen degradability of fresh herbage: effect of maturity and growth type, and prediction from chemical composition and by near infrared reflectance spectroscopy. *Anim. Feed Sci. Technol.* **1992**, *38*, 335–349.

15. Chudnovsky, A.; Ben-Dor, E.; Paz, E. Using NIRS for rapid assessment of sediment dust in the indoor environment. *J. Near Infrared Spectroscopy* **2007**, *15*, 59–70.
16. Chabrillat, S.; Ben-Dor, E.; Viscarra Rossel, R.A.; Demattê, J.A.M. Quantitative soil spectroscopy. *Appl. Environ. Soil Sci*. **2013**, doi:10.1155/2013/616578.
17. Curran, P.J.; Kupiec, J.A.; Smith, G.M. Remote sensing the biochemical composition of a slash pine canopy. *IEEE Trans. Geosci. Remote Sens.* **1997**, *35*, 415–420.
18. Peterson, D.L.; Aber, J.D.; Matson, P.A.; Card, D.H.; Swanberg, N.; Wessman, C.; Spanner, M. Remote sensing of forest canopy and leaf biochemical contents. *Remote Sens. Environ.* **1988**, *24*, 85–108.
19. Wessman, C.A. Remote sensing and the estimation of ecosystem parameters and functions. In *Imaging Spectrometry—A Tool for Environmental Observations;* Hill, J.; Megier, J., Eds.; Kluwer: Dordrecht, the Netherlands, 1994; pp. 39–56.
20. Yoder, B.J.; Pettigrew-Crosby, R.E. Predicting nitrogen and chlorophyll content and concentrations from reflectance spectra (400–2500 nm) at leaf and canopy scales. *Remote Sens. Environ.* **1995**, *53*, 199–211.
21. Marten, G.C.; Shenk, J.S.; Barton, F.E., II. *Near-Infrared Reflectance Spectroscopy (NIRS): Analysis of Forage Quality*; United States Department of Agriculture Handbook 643; USDA: Washington, DC, USA, 1985; pp. 1–96.
22. Norris, K.H.; Barners, R.F.; Moore, J.E.; Shenk, J.S. Predicting forage quality by infrared reflectance spectroscopy. *J. Anim. Sci*. **1976**, *43*, 889–897.
23. Garcia, J.; Cozzolino, D. Use of near infrared reflectance (NIR) spectroscopy to predict chemical composition of forages in broad-based calibration models. *Agricultura Técnica* **2006**, *66*, 41–47.
24. Landau, S.; Friedman, S.; Brenner, S.; Bruckental, I.; Weinberg, Z.G.; Ashbell, G.; Hen, Y.; Dvash, L.; Leshem, Y. The value of safflower (*Carthamus tinctorius*) hay and silage grown under Mediterranean conditions as forage for dairy cattle. *Livestock Prod. Sci*. **2004**, *88*, 263–271.
25. Landau, S.; Glasser, T.; Dvash, L. Monitoring nutrition in small ruminants by aids of near infrared spectroscopy (NIRS) technology: A review. *Small Ruminant Res*. **2006**, *61*, 1–11.
26. Landau, S.; Nitzan, R.; Barkai, D.; Dvash, L. Excretal near infrared reflectance spectrometry to monitor the nutrient content of diets of grazing young ostriches (*Struthio camelus*). *South African J. Anim. Sci*. **2006**, *36*, 248–256.
27. Landau, S.; Giger-Reverdin, S.; Rapetti, L.; Dvash, L.; Dorleans, M.; Ungar, E.D. Data mining old digestibility trials for nutritional monitoring in confined goats with aids of fecal near infra-red spectrometry. *Small Ruminant Res*. **2008**, *77*, 146–158.
28. Lugassi, R.; Chudnovsky, A.; Zaady, E.; Dvash, L.; Goldshleger, N. Spectral slope as an indicator of pasture quality. *Remote Sens.* **2015**, *7*, 256–274.
29. Decruyenaere, V.; Lecomte, P.; Demarquilly, C.; Aufrere , J.; Dardenne, P.; Stilmant, D.; Buldgen, A. Evaluation of green forage intake and digestibility in ruminants using near infrared reflectance spectroscopy (NIRS): Developing a global calibration. *Anim. Feed Sci. Technol.* **2009**, *148*, 138–156.
30. Cozzolino, D. Use of infrared spectroscopy for in-field measurement and phenotyping of plant properties: instrumentation, data analysis, and examples. *Appl. Spectroscopy Rev.* **2014**, *49*, 564–584.

31. Petisco, C.; García-Criado, B.; García-Criado, L.; Vázquez-de-Aldana, B.R.; García-Ciudad, A. Quantitative analysis of chlorophyll and protein in alfalfa leaves using fiber-optic near-infrared spectroscopy. *Comm. Soil Sci. Plant Anal.* **2009**, *40*, 2474–2484.
32. Cozzolino, D.; Labandera, M. Determination of dry matter and crude protein contents of undried forages by near-infrared reflectance spectroscopy. *J. Sci. Food Agric.* **2002**, *82*, 380–384.
33. Dardenne, P.; Agneessens, R.; Sinnaeve, G. Fresh forage analysis by near infrared spectroscopy. In *Near Infrared Spectroscopy: The Future Waves*; Davies, A.M.C., Williams P., Eds.; NIR Publications: Chichester, UK; 1996; pp. 531–536.
34. De la Roza, B.; Martínez, A.; Modroño, S.; Santos, B. Determination of the quality of fresh silages by near infrared reflectance spectroscopy. In *Near Infrared Spectroscopy: The Future Waves*; Davies, A.M.C.; Williams, P., Eds.; NIR Publications: Chichester, UK; 1996; pp. 537–541.
35. Pullanagari, R. Proximal Sensing Techniques to Monitor Pasture Quality and Quantity on Dairy Farms. Ph.D. Thesis, Massey University, Manawatu, New Zealand, 2011.
36. Pullanagari, R.; Yule, I.; Tuohy, M.; Hedley, M.; Dynes, R.; King, W. In-field hyperspectral proximal sensing for estimating quality parameters of mixed pasture. *Precision Agric.* **2012**, *13*, 351–369.
37. Pullanagari, R.; Yule, I.; Tuohy, M.; Hedley, M.; Dynes, R.; King, W. Multi-spectral radiometry to estimate pasture quality components, *Precision Agric.* **2012**, *13*, 442–456.
38. Israel Forecast. Available online: http://www.ims.gov.il/IMSEng/ (accessed on 14 June 2015).
39. Stern, A.; Gradus, Y.; Meir, A.; Krakover, S.; Tsoar, H. *Atlas of the Negev*; Ben Gurion University of the Negev: Beer-Sheva, Israel, 1986.
40. Dan, J.; Yaalon, D.; Kundzimzinsky, H.; Raz, Z. *The Soil of Israel*. ARO Publication Bulletin No. 168 (in Hebrew); Agricultural Research Organization: Bet Dagan, Israel, 1977.
41. Zaady, E.; Levacov, R.; Shachak, M. Application of the herbicide, Simazine, and its effect on soil surface parameters and vegetation in a patchy desert landscape. *Arid Land Res. Manage.* **2004**, *18*, 397–410.
42. Feinbrun-Dothan, N.; Danin, A. *Analytical Flora of Eretz-Israel*; Cana Publishers: Jerusalem, Israel, 1991.
43. Zaady, E.; Yonatan, R.; Shachak, M.; Perevolotsky, A. The effects of grazing on abiotic and biotic parameters in a semiarid ecosystem: a case study from the northern Negev desert, Israel. *Arid Land Res. Manage*. **2001**, *15*, 245–261.
44. Henkin, Z.; Landau, S.; Ungar, E.D.; Perevolotsky, A.; Yehuda, Y.; Sternberg, M. Effect of timing and intensity of grazing on the herbage quality of a Mediterranean rangeland. *J. Anim. Feed Sci*. **2007**, *16*, 318–322.
45. Henkin, Z.; Ungar, E.D.; Dvash, L.; Perevolotsky, A.; Yehuda, Y.; Sternberg, M.; Voet, H.; Landau, S.Y. Effects of cattle grazing on herbage quality in a herbaceous Mediterranean rangeland. *Grass Forage Sci*. **2011**, *66*, 516–525.
46. Tilley, J.M.A.; Terry, R.A. A two-stage technique for the *in vitro* digestion of forage crops. *J. Br. Grassl. Soc*. **1963**, *18*, 104–111.
47. Van Soest, P.J.; Robertson, J.B.; Lewis, B.A. Methods for dietary fiber, neutral detergent fiber and non-starch polysaccharides in relation to animal nutrition. *J. Dairy Sci*. **1991**, *74*, 3583–3597.

48. Kokaly R.F.; Clark R.N. Spectroscopic determination of leaf biochemistry using band-depth analysis of absorption features and stepwise multiple linear regression. *Remote Sens. Environ.* **1999**, *67*, 267–287.
49. Kokaly, R.F. Investigating a physical basis for spectroscopic estimates of leaf nitrogen concentration. *Remote Sens. Environ*. **2001**, *75*, 153–161.
50. Mutanga, O.; Skidmore, A.K. Narrow band vegetation indices overcome the saturation problem in biomass estimation. *Int. J. Remote Sens.* **2004**, *25*, 3999–4014.
51. Noomen, M.F.; Skidmore, A.K.; van der Meer, F.D.; Prins, H.H.T. Continuum removed band depth analysis for detecting the effects of natural gas, methane and ethane on maize reflectance. *Remote Sens. Environ*. **2006**, *105*, 262–270.
52. Esbensen, K. *Multivariate Data Analyses in Practice—An Introduction to Multivariate Data Analyses and Experimental Design*; Aalborg University, CAMO: Esbjerg, Denmark, 2002.
53. Wold, S.; Sjostrom, M.; Eriksson, L. PLS-regression: A basic tool of chemometrics. *Chemometrics Intelligent Lab. Syst.* **2001**, *58*, 109–130.
54. Goldshleger, N.; Chudnovsky, A.; Ben-Binyam, R. Predicting salinity in tomato using soil reflectance spectra. *Int. J. Remote Sens.* **2013**, *34*, 6079–6093.
55. Jensen, J.R. Remote sensing of vegetation. In *Remote Sensing of the Environment: An Earth Resource Perspective*; Prentice Hall: Upper Saddle River, NJ, USA, 2007; pp. 355–408.
56. Clark, R.N.; Roush, T.L. Reflectance spectroscopy: quantitative analysis techniques for remote sensing applications. *J. Geophys. Res.* **1984**, *89*, 6329–6340.
57. Duckworth, J. Mathematical data processing. In *Near-Infrared Spectroscopy in Agriculture*; Roberts, C.A., Workman, J., Jr., Reeves, J.B., Eds.; American Society of Agronomy, Crop Science Society of America, Soil Science Society of America: Madison, WI, USA, 2004; pp. 115–132.
58. Mark, H. Quantitative spectroscopic calibration. In *Encyclopedia of Analytical Chemistry: Applications, Theory, and Instrumentation*; Meyers, R.A., Ed.; John Wiley & Sons Ltd.: Chichester, UK, 2000; pp. 13587–13605.
59. Guo, X.; Wilmshurst, J.F.; Li, Z. Comparison of laboratory and field remote sensing methods to measure forage quality. *Int. J. Environ. Res. Public Health* **2010**, *7*, 3513–3530.
60. Starks, P.J.; Coleman, S.W.; Phillips, W.A. Determination of forage chemical composition using remote sensing. *J. Range Manage*. **2004**, *57*, 635–640.
61. Zhao, D.; Starks, P.J.; Brown, M.A.; Phillips, W.A.; Coleman, S.W. Assessment of forage biomass and quality parameter of bermudagrass using proximal sensing of pasture canopy reflectance. *Grassland Sci.* **2007**, *53*, 39–49.
62. Adjorlolo, C.; Mutanga, O.; Cho, M.O. Predicting C3 and C4 grass nutrient variability using *in situ* canopy reflectance and partial least squares regression, *Int. J. Remote Sens.* **2015**, *36,* 1743–1761.