NEAR INFRARED REFLECTANCE SPECTROSCOPY – A TOOL TO ESTIMATE N_2-FIXATION

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ABSTRACT

Legume content is a main factor to describe the amount of N_2-fixed in a multi-species legume-grass-mixture. In this study, Fourier-Transform Near Infrared Reflectance Spectroscopy (FT-NIRS) was used to estimate the legume content of oven dried (60°C) ground (1,5 mm) samples to gather information about the spatial variability of N_2-fixation. The FT-NIRS was calibrated with handselected grass and legume samples combined with artificial mixtures to represent the legume content in between (5% increments). Calibration statistics (one leave out cross validation) showed a high coefficient of determination ($R^2 = 99\%$) and a small prediction error (RMSECV = 3%). This calibration model was applied to field samples (n = 90, field size 2,25 ha). The obtained legume content data were used as a factor in the calculation of N_2-fixation. For the one shown harvest N_2-fixation varied in a wide range from 3 to more than 11 g N/m².

INTRODUCTION

The amount of N_2 fixed in legume-grass mixtures is mainly influenced by yield and legume content (WEIßBACH 1995, BOLLER 1988). N_{fixed} can be calculated as shown in equation [1].

$$N_{fixed}\,[g/m^2] = Yield\,[g/m^2] * Leg\,[\%] * N_{leg}[\%] * N_{dfA}\,[\%] \quad \text{[eq. 1]}$$

where

Yield	is the dry-matter yield of the legume grass mixture
Leg	is the legume content of the mixture (on a DM basis)
N_{leg}	is the nitrogen content of the legume fraction
N_{dfA}	is the nitrogen derived from atmosphere in % of the legume-N

The spatial variability of N_2-fixation has not been described yet, although it is supposed to be an important factor for calculating accurate N-balances. This is due to several problems when working on the field scale. First, there is no adequate procedure for the measurement of yield with a sufficient accuracy and spatial resolution apart from laborious manual weighing. Secondly, the other supposed main factor, legume content, can only be estimated by eye. Visual estimates are subjective and involve high errors and low repeatability. On the other hand highly accurate but time consuming botanical analyses are not suitable for spatial studies. Therefore a fast and accurate assessment of the legume content is essential for the description of the spatial variability of N_2-fixation. NIRS has been described to be a promising tool for the fast determination of the botanical composition of legume-grass mixtures (COLEMAN 1985, SHAFFER 1990). In general, NIRS methods use the intensity of the reflectance of a sample in the region of near infrared radiation (12500-4000 cm^{-1}). Reflectance values are continuously detected (determined by the resolution used) and a NIR spectrum is generated. The structure of the spectrum characterizes the components of the sample. For the prediction of the concentration of certain components the calibration of the NIRS is crucial. Therefore the quality of the calibration will determine the precision of the prediction of the legume content. For the calibration of the NIRS, the known concentration values of the calibration samples (= standards) are assigned to the spectra. Using multivariate statistics, a calibration algorithm is set up, which finally allows to determine the unknown concentration of the constituent in a sample.

In our project a multivariate calibration was developed, that is suitable for the measurement of legume content of multi-species-mixtures. This allows a more accurate description of the spatial variability of N_2-fixation than a visual estimation of the legume content. Additionally it was tested whether N_{leg} and N_{dfA} can be used as constant parameters for describing spatial variability of N_2-fixation.

MATERIAL AND METHODS

In 1999 experiments were run with a multi-species legume-grass mixture (Medicago sativa, Trifolium pratense, Trifolium repens, Dactylis glomerata, Festuca pratense, Arrhenaterum elatior) on field A09 (2,25 ha) of the FAM Research Station Scheyern, Germany. Spatial variability of N_2-fixation was described by measurements of yield and legume content. Yield was measured on 90 plots each 12 m² in size. At each harvest (1^{st}: 18.05.99; 2^{nd}: 16.07.99; 3^{rd}: 26.08.99; 4^{th}: 07.10.99) dry matter yield (60°C, 48h) was assessed by a representative subsample. The dried subsamples were ground in a mill (BRABENDER, Duisburg, Germany) to pass a 1,5 mm screen and the legume content was measured with FT-NIRS (Vector 22/N, BRUKER, Ettlingen, Germany). The spectra were measured with a resolution of 10 cm^{-1} and 30 scans per sample in the range from 10000 to 3500 cm^{-1}. The FT-NIRS was calibrated with handsorted pure grass and legume samples taken at each harvest and additionally with artificial mixtures of grass and legume samples varying legume contents in 5% increments up to 95% (411 calibration samples). Data pre-treatment of the spectra and the calculation of the Partial Least Square Regression (PLS) for the multivariate calibration were performed with a chemometrical software (OPUS 2.08, BRUKER, Ettlingen, Germany). N_{leg} and N_{dfA} were measured on three subplots (at least six replicates) which represented the heterogenity of the field. The assessment of N_{dfA} was performed with isotope dilution method. For the calculation of N_2-fixation by equation [1] NIRS based legume content was used. All data were interpolated (inverse distant weighing) with ArcView 3.1 GIS (ESRI, New York, USA) in order to describe the spatial variability of N_2-fixation.

RESULTS AND DISCUSSION

1. NIRS calibration

Vector normalization and first derivative were used as pre-treatment of the spectra for the development of an optimal prediction model with PLS. The predicted values of legume content were highly correlated with the true values of the standards (Figure 1 A). Calibration statistics showed a high coefficient of determination ($R^2 = 99\%$) and a low prediction error (RMSECV = 3%). Ten factors were used in the PLS algorithm to explain the variance of the spectral data (MARTENS 1989). Though a rather high R^2 was already achieved at the 4th factor, the RMSECV could be improved by increasing the number of factors up to ten (Figure 1 B). To test its general applicability, the calibration must be validated with an independent set of validation samples (different phenological stages, different fields, harvesting times).

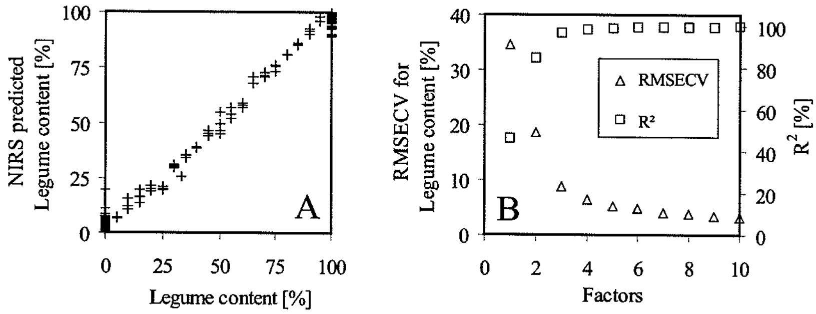

FIGURE 1. NIRS-calibration statistics: Legume content as predicted by the NIRS model vs. true values of the artificially mixed standards, n = 411 (A). Course of prediction error (RMSECV) and coefficient of determination (R^2) with increasing number of factors in the PLS algorithm (B).

2. Variability of yield, legume content and N_2-fixation

In 1999 yield and legume content were assessed four times (Table 1). DM-yield was highest at harvest 2 whereas mean legume content increased until harvest 3 from 55% to 73%. N_{leg} and N_{dfA} varied from harvest to harvest, but both showed minor variation (except N_{dfA} at harvest 4) in the field. This observation justified to use them as constant factors in equation [1]. Therefore only yield and legume content contributed to the spatial variability of N_2-fixation within one field at one harvest. Both varied in a wide range and high values of yield did not necessarily reflect high percentages of legumes in the mixture and vice versa (Figure 2). It was concluded that the pattern of N_2-fixation was only described precisely, if both, total dry matter yield and legume content were included in the calculation. The variability caused a wide range of N_2-fixation at each harvest.

TABLE 1. Yield parameters of the legume-grass mixture of field A09 and calculated N_2-fixation at four harvests in 1999. Yield, legume content and N_2-fixation were assessed for the whole field (n=90), whereas N_{leg} and N_{dfA} were measured at three subplots with at least six replicates.

Harvest	Yield		Legume content		N_2-fixation		N_{leg}		N_{dfA}	
No.	$\bar{x}$	s	$\bar{x}$	s	$\bar{x}$	s	$\bar{x}$	s	$\bar{x}$	s
	---- [g/m²] -- --		----- [%] ----- -		---[g N/m²] ---		-------------- [%] --------------			
1	368	77	6,2	2,3	55	15	3,2	0,2	95	1,8
2	452	105	6,6	2,1	65	12	2,4	0,2	95	2,5
3	348	70	7,9	2,0	73	11	3,4	0,1	91	4,9
4	103	38	2,9	1,3	72	15	4,1	0,3	86	10

$\bar{x}$ = mean, s = standard deviation

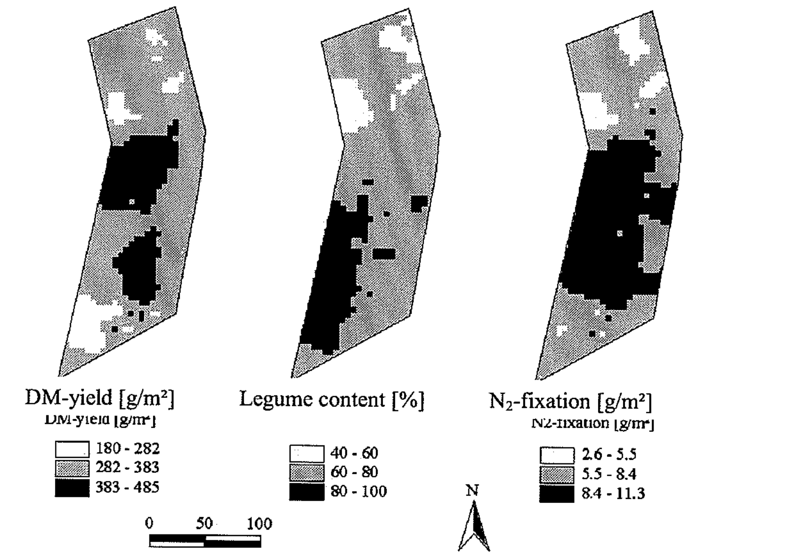

FIGURE 2. Yield, legume content predicted by NIRS and calculated N_2-fixation of the

CONCLUSION

NIRS is a promising tool for measuring easily and precisely legume content in a multi-species mixture. It could be shown for the first time, that N_2-fixation of a legume-gras-mixture varies strongly within one field. This differentiation was caused by the different patterns of both DM-yield and legume content. Therefore the assessment of DM-yield is not sufficient to describe variation of N_2-fixation but legume content has to be taken into account. The presented NIRS method enables one to measure the legume content and to describe more precisely the spatial variability of the N_2-fixation. Still, its general applicability has to be proven by broadening the variation of the included samples.

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