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Field scale variability of N_2-fixation in legume and grass mixtures

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Abstract

Spatial variability of N_2-fixation of a legume-grass mixture has not been previously described. 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 get information about the spatial variability of N_2-fixation. The FT-NIRS was calibrated with 411 calibration samples. The cross validation of the calibration model showed a high coefficient of determination ($R^2 = 0.99$) and a small prediction error (RMSECV = 3%). In a second step 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 one harvest N_2-fixation varied in a wide range from 3 to more than 11 g N m^{-2} within one field.

Introduction

The amount of N_2 fixed in legume-grass mixtures is mainly influenced by yield and legume content (Weißbach *et al.* 1995, Boller 1988). N_{fixed} can be calculated as shown in Equation [1].

Equation [1]

$$N_{fixed}\,[g\ m^{-2}] = Yield\,[g\ m^{-2}] * Leg\,[\%] * N_{leg}\,[\%] * N_{dfA}\,[\%]$$

where:-

Yield	is the dry matter (DM) 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 proportion of nitrogen derived from the atmosphere of the total legume-N

The spatial variability of N_2-fixation has not been previously described, although it is supposed to be an important factor for calculating accurate N-balances. This is due to several problems when working on 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 *et al.* 1985, Shaffer *et al.* 1990).

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 by 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 the spatial variability of N_2-fixation.

Materials 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 m^2 in size. At each harvest (1^{st}: 18^{th} May 1999; 2^{nd}: 16^{th} July 1999; 3^{rd}: 26.th August 1999; 4^{th}: 7 th October 1999) 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 FT-NIRS was calibrated with hand-sorted pure grass and legume samples taken at each harvest. Additionally artificial mixtures of grass and legume samples, varying in legume contents of 5% increments up to 95% were used in calibration (411 calibration samples). N_{leg} and N_{dfA} were measured on three sub-plots (at least six replicates) which represented the heterogeneity of the field. N_{dfA} was measured with ^{15}N isotope dilution method (Warembourg 1993). For the calculation of N_2-fixation by Equation [1] NIRS based legume content was used. All data were interpolated (inverse distant weighting) with ArcView 3.1 GIS (ESRI, New York, USA) in order to describe the spatial variability of N_2-fixation.

Results and discussion

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%.

Table 1. Yield parameters and calculated N_2-fixation of a clover-grass mixture of field A09 at four harvests in 1999.

Harvest	Yield		Legume content		N_2-fixation	
No.	M	s	M	s	M	s
	--- [g m^{-2}] ---		----- [%] ------		---[g N m^{-2}] ---	
1	368	77	55	15	6.2	2.3
2	452	105	65	12	6.6	2.1
3	348	70	73	11	7.9	2.0
4	103	38	72	15	2.9	1.3

M= mean, s = standard deviation
The data were assessed for the whole field (n=90)

N_{leg} and N_{dfA} varied from harvest to harvest, but both showed negligible variation in the field except for N_{dfA} at harvest 4 (Table 2).

Table 2. N content (N_{leg}) and N derived from the atmosphere (N_{dfA}) of the legume fraction at four harvests in 1999

Harvest	N_{leg}		N_{dfA}	
No.	M	s	M	s
	--------------- [%] ---------------			
1	3.2	0.2	95	1.8
2	2.4	0.2	95	2.5
3	3.4	0.1	91	4.9
4	4.1	0.3	86	10

M= mean, s = standard deviation
Data were assessed in three plots with at least six replicates

This observation justified the use of 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. High values of yield did not necessarily reflect high percentages of legumes in the mixture and vice versa (Figure 1). It was concluded that the pattern of N_2-fixation was only described precisely, if both, total DM-yield and legume content were included in the calculation. The variability caused a wide range of N_2-fixation at each harvest. Although both parameters varied strongly at each harvest on the field scale, the pattern was quite similar to the harvest shown in Figure 1.

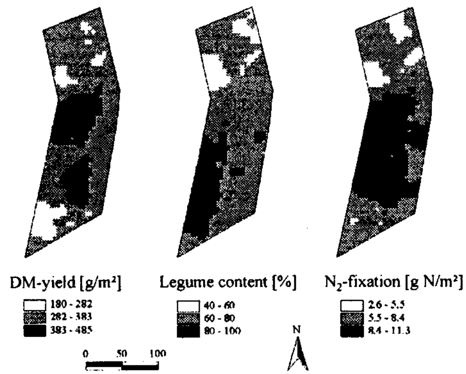

Figure 1. Yield, legume content predicted by NIRS and calculated N_2-fixation of the legume-grass mixture at harvest 3 of field A09 in 1999.

Conclusions

It could be shown for the first time, that N_2-fixation of a legume-grass-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, legume content has to be taken into account as well. NIRS is a promising tool for easily and precisely measuring legume content in multi-species mixtures. The general applicability of the calibration has to be examined by broadening the variation of the included samples.

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