Methodological aspects of determination of biological N-fixation of different forage legumes.

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Abstract
Knowledge about the amount of fixed nitrogen of different legume crops is very important for calculation of farm N balances. According to literature the choice of determination method may have an impact on the estimated amount of N fixed by a legume sward. The aim of the study was to compare the three most important field methods for determination of nitrogen fixation under different sward management systems. In the present study the natural 15N abundance method gave lower fixation rates than the two alternative methods (total-N-difference method and 15N enrichment technique). The determination of N fixation based only on N in harvestable plant material underestimated the amount of fixed N on average by 70 kg · ha^{-1} compared to techniques including also the amount of N in non harvestable plant parts.

keywords : biological N-fixation, 15N abundance method, 15N enrichment technique, red clover, alfalfa, white clover

Introduction
Not renewable energy sources are limited. This will lead to higher prices for mineral N fertilisers in the long term. The application of strategies for biological N-fixation may be an alternative. Especially in forage production systems legumes can be used for N fixation. Nitrogen fixation of a forage legume sward is strongly depending on legume species, companion grass species, seed mixture and management of the sward.
The amount of fixed nitrogen by legumes, the pathways of N fixation into forage yield, amount of N in the crop residues, enrichment of soil-N and yield of the following crop is needed for calculation of farm N balances as well as to design crop rotations. The main critical point is that the choice of determination method may have an impact on the estimated amount of N fixed by a legume sward. (Shearer and Kohl, 1986; Ledgard and Steele, 1992; Loges, 1998). Systematic comparisons of estimation methods based on a wide range of different managed legume are scarce. The aim of the presented study was to compare the three most important field methods for determination of nitrogen fixation under different sward management systems.

Materials and methods
A field experiment was carried out from 1994-99 on a sandy, loamy cambisol (14% clay) at the experimental station Hohenschulen near Kiel in Northern Germany (9°37'E, 54°21'N; average annual precipitation: 716 mm; mean annual temperature: 7,8 °C). The experiment included the following factors and treatments (i) legume species (treatments: a) red clover, b) alfalfa, c) white clover), (ii) seed mixture (treatments: a) pure legume, b) mixture with perennial ryegrass) and (iii) management (treatments: a) forage use, b) green manure). In each of the possible factor combinations measurements of biomass and N amount of harvestable and not harvestable plant parts were performed. To quantify methodological effects, biological N-fixation was estimated using three different methods (total-N-difference method, natural ^{15}N abundance method, 15N enrichment technique) simultaneously.
In addition to this, each of the chosen methods was carried out in a simple and an extended version. While in the simple version the determination of N-fixation was based on only N in

the harvestable plant material, considered the extended calculation method on top of this also the N amount in not harvested plant parts as well as CaCl2-extractable soil-N fractions. For each legume sward an unfertilised pure perennial ryegrass sward under a similar management system was used as reference plants for estimation of N-fixation.

Results and discussion

Figure 1 shows the highly significant interactions of legume species, seed mixture and management on N-fixation. All cropped swards fixed higher amounts of N than the mulched ones. With an average of 330 kg N ha^{-1} the cropped swards with alfalfa or red clover fixed at least 50 kg N ha^{-1} more than the cropped swards with white clover. In the mulched green manure system the swards with white clover reached higher fixation rates than the swards with the other two legume species.

In Table 1 results of different methods for N-fixation estimation are compared with respect to the experimental factors seed mixture and management. The determination of N-fixation based on only N in harvestable plant material underestimates the amount of fixed N on average by 70 kg ha^{-1} compared to techniques including also soil mineral N and N in non harvestable plant parts. While N difference method and ^{15}N enrichment technique gave comparable estimations, were results obtained by natural ^{15}N abundance significantly lower. For the parameter N-fixation, the choice of method slightly affected the ranking between the experimental treatments. As reasons for the differences between estimations obtained by the natural ^{15}N abundance and the results of the other two methods the following factors have to be taken into account: a) a quite low natural soil 15N enrichment of the experimental site (only 3.5 $\delta^{15}N$ ‰ instead of the demanded 6 $\delta^{15}N$ ‰ (Peoples et al.,1991)), b) a distinct spatial variation of the natural soil 15N enrichment between the plots, c) immobilisation of mineral soil-N under the not fertilised pure perennial ryegrass swards which were used as reference crops for estimation of N-fixation.

Conclusions

N-fixation of forage legume swards can be varied in a wide range through different combinations of the factors legume species, seed mixture and management system. The choice of method has a strong effect on the determined N fixation of a legume sward. Comparing studies using different methods this has to be taken into account. The determination of N fixation of grass/legume mixtures in the first production year based only on N in the harvested plant material underestimates the amount of fixed N considerably compared to techniques including also N in non harvestable plant parts.

Perspectives

All tested estimation methods are very expensive and time consuming and therefore have to be evaluated more as scientific instruments than as tools for farm scale N balances. It was therefor also aim of the project to find relationships between N-fixation and parameters that are easy to estimate also by farmers like either sward legume content DM or legume DM-yield. As an example table 2 shows for different grass/red clover swards used for forage in the first production year highly significant dependencies between N-fixation on the one side and clover content in DM yield or clover yield on the other side. Especially the relationship between clover-yield and N-fixation can be considered as a starting point for empirical models for estimation of nitrogen fixation like the Danish model proposed by Høgh-Jensen *et al.* (1998).

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Table 1. Effect of method on estimation of N-fixation (kg N $ha^{-1} \cdot year^{-1}$) of different grass/legume swards (1997, average of three legume species)

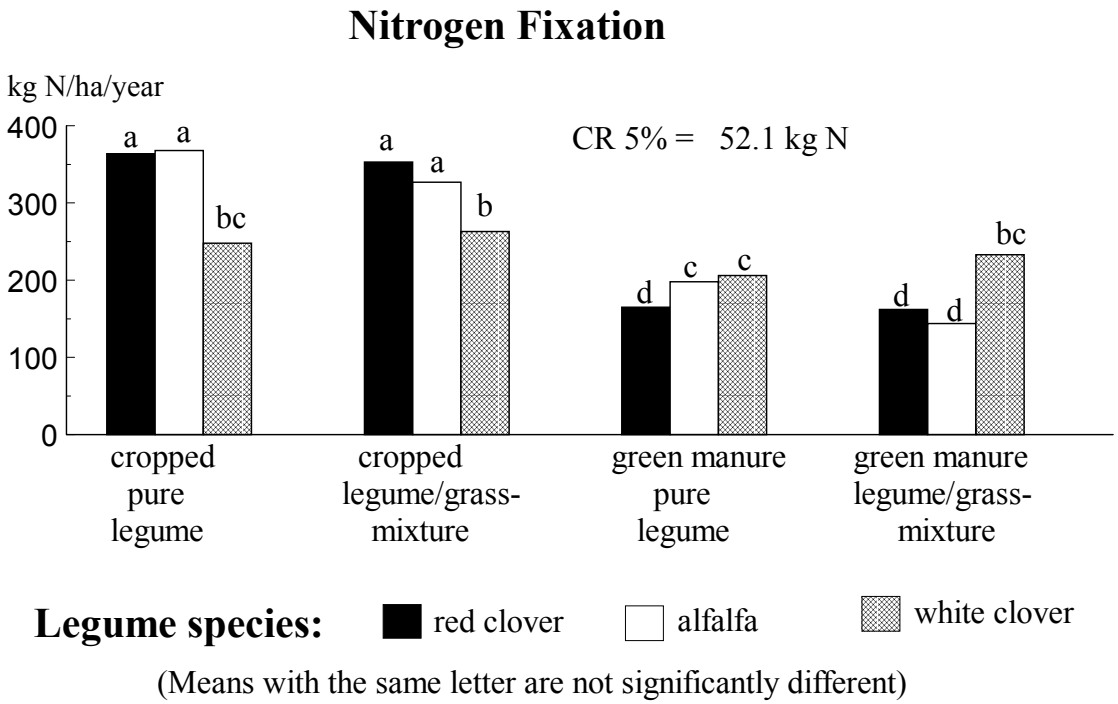
Seed mixture (management)	based on N in harvestable plant material			based on total N		
	N difference	^{15}N enrich-ment.	natural ^{15}N abundance	N-difference	^{15}N enrich-ment	natural ^{15}N abundance
Pure legume (forage use)	274.0a	285.0a	168.5a	315.2a	354.9a	248.5a
Grass/legume (forage use)	259.8b	235.8b	159.0a	303.8a	291.9b	215.8b
Pure legume (green manure)	100.4c	92.8c	52.7b	178.4b	196.8c	172.5c
Grass/legume (green manure)	90.8c	80.9d	47.7b	166.4b	168.2d	111.0d

Data in same column with similar letters do not differ at $p<0.05$ level, Student-Newman-Keuls-Test

Table 2. Linear relationship between either clover content in DM yield [% of DM] (x_1) or clover yield [t DM $\cdot$ $ha^{-1} \cdot year^{-1}$] (x_2) and N-Fixation (y) (estimated with the N-difference method based on total N [kg N $\cdot$ $ha^{-1} \cdot year^{-1}$] of grass/red clover swards used for forage in the first production year (average of 1994 and 1995) (se: standard error, n = 40, scope: x_1: 27 - 100 % of DM, x_2: 3.7 – 13.4 t DM $\cdot$ $ha^{-1} \cdot year^{-1}$)

Dependent variable	Equation	Independent variable	S	r^2
N-Fixation (y)	$y = 155.6141 + 2.21986x_1$	Clover content (x_1))	46.6	0.58***
N-Fixation (y)	$y = 128.6178 + 21.4744x_2$	Clover yield (x_2)	32.5	0.80***

Figure 1. Effect of management, legume species and seed mixture of grass/legume on N-fixation (kg N $ha^{-1}.year^{-1}$) in 1997. (Calculation method: total-N-difference method based on N-amounts in harvestable and not harvestable plant material, under consideration of the CaCl2-extractable soil-N fractions)



(Means with the same letter are not significantly different)

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