

# How to optimise symbiotic nitrogen fixation in organic crop rotations

*Knut Schmidtke*

Hochschule für Technik und Wirtschaft Dresden (FH), University of Applied Sciences, Pillnitzer Platz 2, D-01326 Dresden, Germany, e-mail: [schmidtk@pillnitz.htw-dresden.de](mailto:schmidtk@pillnitz.htw-dresden.de)

## 1 Introduction

Nitrogen discharges of an ecologically operated farm, which primarily result from the sales of vegetable and animal products, can only be substituted to a limited extent in ecological gardening and cultivation through the supply of organic fertiliser. Therefore, it is important to integrate legumes into the crop rotation in order to assure high nitrogen provision of the plant cultivation system. Plant cultivation strategies in ecological agriculture therefore also need to focus on extracting high amounts of symbiotic fixed nitrogen within the phase of legumes cultivation. The most important agricultural strategies for optimisation of the symbiotic  $N_2$  fixing activity of legumes should be illustrated here. Furthermore, in order to plan the nitrogen supply in legumes based crop rotations, not only knowledge about the amount of symbiotic fixed nitrogen is necessary but also knowledge about the height of the N balance which has to be listed with the cultivation of legumes must be given. Therefore, the first part of the article explains which nitrogen flows need to be captured and considered in the N balance in order to detect the overall amount of symbiotic fixed nitrogen by legumes.

## 2 Nitrogen flows during the cultivation of legumes

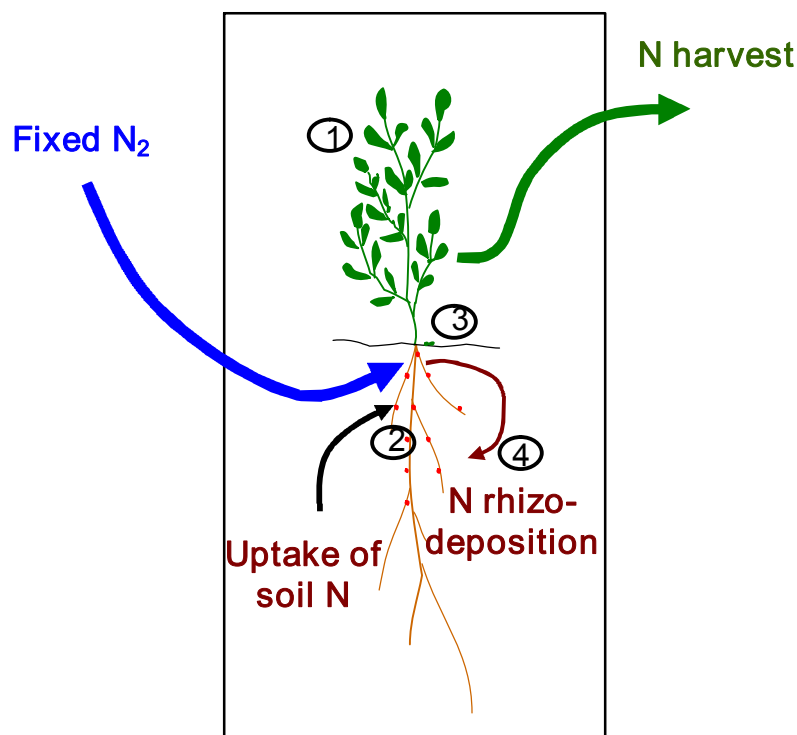


Figure 1: Nitrogen flows during the cultivation of legumes

At harvest time of pure seed legumes the symbiotic fixed nitrogen is contained in the shoot (1), in the roots (2) and in the litterfall (3) as well as having already reached the soil through rhizodeposition (4) during the growth of the legume (Figure 1). This overall amount N symbiotically fixed by the legume counts as supply N, i.e. the amount N departed with the harvest crop (N in grain or herbage), as removal into the N balance (Figure 1). Table 1 shows the N amounts of shoot and roots, which were detected during the cultivation of pea, faba bean, red clover and lucerne in a field experiment on a fluvisol near Goettingen (Germany). The N amounts contained in the roots of legumes are frequently not detected in field experiments for detection of symbiotic N<sub>2</sub> fixation activity (Jensen 1996a). Nevertheless, they are very relevant for the nitrogen supply in legume based crop rotations, as shown by the results of Table 1: between 4.7% (faba bean) and 19.3% (lucerne) of the overall N amount of legumes located in the shoot and roots could be found in the roots (Table 1).

**Table 1:** Grain and herbage yield [t ha<sup>-1</sup>] and amount of nitrogen [kg ha<sup>-1</sup>] in shoot and roots of organically grown legume species in a field experiment near Goettingen (Germany)

	Pea ( <i>Pisum sativum</i> )	Faba bean ( <i>Vicia faba</i> )	Red clover ( <i>Trifolium pratense</i> <sup>1)</sup> )	Lucerne ( <i>Medicago sativa</i> <sup>1)</sup> )
Grain yield	5.39	5.68		
Herbage yield			9.05	8.03
N in grain	184.3	281.2		
N in herbage			273.6	277.4
N in straw (and litter)				
N in stubble (and litter)	52.2	79.8	30.6	19.1
Roots	14.8	17.7	54.6	71.1
<u>In shoot and roots</u>				
N derived from:				
Symbiotic N <sub>2</sub> fixation <sup>5)</sup>	218.4	341.0	284.2	291.2
Soil	32.9	37.7	74.6	76.4
N harvest index	0.733	0.743	0.763	0.754
Fixed N <sub>2</sub> in rhizodeposits	28.2 <sup>2)</sup>	44.8 <sup>3)</sup>	14.5 <sup>4)</sup>	22.1 <sup>4)</sup>
N balance <sup>6)</sup>	+ 63,3	+ 104.6	+ 25.1	+ 35.9

<sup>1)</sup> Sown in springtime at the same time like pea and faba bean and grown until the beginning of October (3 cuts), <sup>2)</sup> according to the results from pot experiments listed in Table 2, <sup>3)</sup> according to results of Mayer et al. and Schmidtke listed in Table 2, <sup>4)</sup> according to results of Schmidtke listed in Table 2, <sup>5)</sup> estimated by <sup>15</sup>N natural abundance method (Shearer & Kohl 1986) <sup>6)</sup> including fixed N<sub>2</sub> in rhizodeposits

In the field experiment illustrated here, the symbiotic fixed N amount of legumes has been estimated by means of the natural <sup>15</sup>N abundance in the soil (Shearer & Kohl 1986). The method is with the existence of a sufficient <sup>15</sup>N abundance in the soil (> 5‰ δ<sup>15</sup>N, Peoples et al. 1989) in particular very appropriate for experiments in ecological agriculture because no <sup>15</sup>N enriched fertiliser has to be used. Should the natural <sup>15</sup>N abundance in the soil at the experiment setting not be high enough, the symbiotic fixed N amount of legume can be

estimated by a large-scale, low-level  $^{15}\text{N}$  application (Reiter et al. 2002a) or the classical  $^{15}\text{N}$ -dilution method (McAuliffe et al. 1958). However, only  $^{15}\text{N}$  enriched organic materials (e.g.  $^{15}\text{N}$  enriched, easily decomposable vegetable biomass) that are approved for Organic Agriculture can be used for this purpose on certified ecologically farmed surfaces.

Furthermore, it has to be acknowledged that already during the growth of legumes nitrogen derived from the symbiotic  $\text{N}_2$  fixation arrive in the soil through rhizodeposition, which have to be allowed for in the N balance (Figure 1). For the estimation of N rhizodeposition of legumes, several indication techniques with  $^{15}\text{N}$ -enriched nitrogen have been developed (split-root technique: Sawatsky & Soper 1991, cotton-wick method: Russell & Fillery 1996,  $^{15}\text{N}$  leaf feeding: Høgh-Jensen & Schjoerring 2001). Here the nitrogen within the root and the nitrogen which is dispensed by the root into the soil are marked with  $^{15}\text{N}$ . In this way nitrogen which has been dispensed by the root into the soil can be detected. With the help of these  $^{15}\text{N}$ -marking techniques it has been detected that – as measured by the harvest overall N-amount in shoot and roots of the legumes - between 5.1% (red clover) and 91.5% (white clover) of this N amount have additionally attained in the soil through rhizodeposition (Table 2).

The types of legumes partially differ significantly in the level of the N rhizodeposition as some experiments have shown (Høgh-Jensen & Schjoerring 2001, Khan et al. 2002, Schmidtke 2005c), which have compared the level of N rhizodeposition of several legumes. Therefore, the level of the N rhizodeposition has to be considered species specific during the calculation of total symbiotic  $\text{N}_2$  fixation activity of legumes and the N balance during the cultivation of legumes. However, some of the results concerning the level of N rhizodeposition, particularly those with high amounts of N rhizodeposition (Table 2), have to be evaluated with reservation, because an exact quantification of N rhizodeposition is knotted with specific requirements (Schmidtke 2005a, Schmidtke 2005b, Mahieu et al. 2007), which could not be fulfilled in some of the experiments mentioned in Table 2. Therefore, especially the results of the field experiments with pea, white clover and red clover distinguish with a significantly higher N rhizodeposition from the results, which have been detected for these plants under controlled conditions in the greenhouse or climate chamber (Table 2).

**Table 2:** N rhizodeposition of different legume species

Species	% N rhizodeposition (of N in shoot and roots) <sup>1)</sup>	Experimental conditions	Reference
<i>Pisum sativum</i>	7.5	Pot	Jensen 1996b
<i>Pisum sativum</i>	14.6	Pot	Mayer et al. 2003
<i>Pisum sativum</i>	22.1	Pot	Schmidtke 2005a
<i>Pisum sativum</i>	9.1	Pot	Schmidtke 2005c
<i>Pisum sativum</i>	12.5	Pot	Schmidtke unpublished
<i>Pisum sativum</i>	57.2	Field	Wichern et al. 2007
<i>Pisum sativum</i>	30.8	Field	Mahieu et al. 2007
<i>Pisum sativum</i>	11.4	Pot	Mahieu et al. 2007
<i>Lathyrus sativus</i>	16.7	Pot	Schmidtke 2005a
<i>Vicia faba</i>	15.5	Pot	Mayer et al. 2003
<i>Vicia faba</i>	10.8	Pot	Schmidtke unpublished
<i>Vicia faba</i>	30.1	Pot	Khan et al. 2002
<i>Cicer arietinum</i>	82.3	Pot	Khan et al. 2002
<i>Vigna radiata</i>	19.8	Pot	Khan et al. 2002
<i>Cajanus cajan</i>	57.6	Pot	Khan et al. 2002
<i>Lupinus angustifolius</i>	22.6	Pot	Russel & Fillery 1996
<i>Lupinus albus</i>	18.8	Pot	Mayer et al. 2003
<i>Lupinus luteus</i>	13.3	Pot	Schmidtke 2005c
<i>Vicia villosa</i>	19.3	Pot	Schmidtke 2005c
<i>Vicia sativa</i>	17.9	Pot	Schmidtke 2005c
<i>Trifolium pratense</i>	55.6	Field	Høgh-Jensen & Schjoerring 2001
<i>Trifolium pratense</i>	5.1	Pot	Schmidtke (unpublished)
<i>Trifolium repens</i>	91.5	Field	Høgh-Jensen & Schjoerring 2001
<i>Medicago sativa</i>	7.6	Pot	Schmidtke unpublished
<i>Trifolium subterraneum</i>	27.3	Pot	McNeill et al. 1998
<i>Ornithopus compressus</i>	28.0	Pot	McNeill et al. 1998

<sup>1)</sup> = (Amount of N rhizo-deposition/(N in shoot and roots)) × 100, according to Schmidtke (2005a)

The cultivation of pea as well as of faba bean was related to a high grain yield (more than 5 t ha<sup>-1</sup>) and low N absorption from the soil (less than 40 kg N ha<sup>-1</sup>) as well as with a high symbiotic N<sub>2</sub> fixation activity and a positive N balance (Table 1). Compared to both grain legumes the N balance was lower during cultivation of red clover and lucerne (Table 1), amongst others because here the forage legumes absorbed more nitrogen out of the soil than the grain legumes. For all four types of legumes with the harvest yield more that 70%

(N harvest index > 0.7, Table 1) of the nitrogen contained in the shoot and roots were taken from the land, so that with increased N absorption from the soil and resulting reduced symbiotic N<sub>2</sub> fixation the cultivation of legumes can also lead to a negative N balance.

Had the legumes illustrated in Table 1 in each case absorbed 60 N ha<sup>-1</sup> more nitrogen from the soil due to a higher amount of plant available nitrogen in the soil and had the symbiotic N<sub>2</sub> fixation in the shoot and roots been decreased to accordingly 60 kg N ha<sup>-1</sup>, the N balance with the same high yield of the legumes would have been declined to - 5.5 kg N ha<sup>-1</sup> (pea), + 36,7 kg N ha<sup>-1</sup> (faba bean), - 38,0 kg N ha<sup>-1</sup> (red clover) and -28,6 kg N ha<sup>-1</sup> (lucerne). Negative N balances during the cultivation of pea have already been detected in ecological agriculture (Corre-Hellou & Crozat 2005, see also Table 4 and Table 6). Therefore, it is important to follow three crop production objectives during the optimisation of symbiotic nitrogen fixation activity of legumes and nitrogen supply in legume based crop rotations:

1. Maximisation of legume yield, because with higher yielding nitrogen demand and simultaneously the symbiotic N<sub>2</sub> fixation activity of the legume increases.
2. Reduction of plant available nitrogen in the soil during the growth of the legume, so that the legume can meet the N demand predominantly from the symbiotic N<sub>2</sub> fixation.
3. Choice of legume types, which leave more nitrogen in the harvest residues on the field (e.g. lower N harvest index, higher N rhizodeposition) or adding a cultivation of legumes for green manuring (N harvest index = 0).

### **3 Optimising symbiotic N<sub>2</sub> fixation**

#### **3.1 Maximisation of crop yield**

Given that with increasing yield the symbiotic N<sub>2</sub> fixation activity of the legumes increases (Köpke 1996), all crop production arrangements in ecological agriculture need to focus on maximising the yield of legumes. Requirement for a good yield of legumes are healthy plant stocks, which are neither strongly attacked by insects (e.g. pea weevil, Corre-Hellou & Crozat 2005) nor by soil-borne diseases. To prevent soil-borne diseases, a widely contrived crop rotation has to be maintained during the cultivation of legumes, which can include between 4 to 5 years of cultivation pauses for some types.

An optimisation of yield of the legumes can particularly be warranted by the choice of location adapted forage or grain legume types, which are able to best exploit the available growth resources under given location conditions. Variety conditional differences in the level of the symbiotic N<sub>2</sub> fixation activity of legumes are available at forage legumes (Jung 2003) as well as grain legumes (Jost 2003), whereas the variety conditional differences between modern varieties are rather small. For the forage legumes cultivation it is required to exploit the vegetation period between precrop and preceding crop completely. As forage legumes in general show a slow youth development with initially low dry matter increase (Frame et al. 1998), an undersown crop in a cover crop instead of a sole crop of the forage legume establishing after harvest of the precrop can help to extend the available vegetation period

with the objective to increase the symbiotic N<sub>2</sub> fixation activity of the forage legume. Long-term cultivated forage legumes are also significantly more effective than yearlong cultivated forage legumes like Jung (2003) shows in a comparison between hardy lucerne and red clover, which were directly sown after the cereal precrop in the beginning of August and the not hardy Persian clover (*Trifolium resupinatum*), which was sown in the same experiment only in the beginning of April the following year. Due to the pre-winter development of red clover and lucerne, these legume types were able to realise a high increase in dry matter already in the early spring of the following year. The Persian clover which was only sown in spring first could not entirely use the resource offer. So the Persian clover could only gain 50% respectively 55% of dry yield in shoot and root and 40% respectively 46% of symbiotic N<sub>2</sub> fixation activity compared to lucerne and red clover (Table 3).

**Table 3:** Dry matter yield (sum of shoot and roots) and amount of nitrogen derived from symbiotic N<sub>2</sub> fixation in shoot and roots of lucerne, red clover and Persian clover during the first production year (Jung 2003)

	Dry matter yield [t DM ha <sup>-1</sup> a <sup>-1</sup> ]	Symbiotic N <sub>2</sub> fixation <sup>3)</sup> [kg N ha <sup>-1</sup> a <sup>-1</sup> ]
Lucerne <sup>1)</sup>	20.7	409
Red clover <sup>1)</sup>	18.6	361
Persian clover <sup>2)</sup>	10.3	165

<sup>1)</sup>sown at the beginning of August before the production year, 4 cuts; <sup>2)</sup>sown at the beginning of April in the production year, 3 cuts, <sup>3)</sup>estimated by <sup>15</sup>N natural abundance method (Shearer & Kohl 1986)

During the grain legume cultivation the competition through weeds can significantly constrict the crop yield and N<sub>2</sub> fixation of legumes. Analyses of ecologically cultivated peas for grain in France have particularly shown a significant decrease of pea yield of more than 1.0 t ha<sup>-1</sup> in weed shoot masses (Corre-Hellou & Crozat 2005). Indeed, here the proportion of nitrogen derived from N<sub>2</sub> fixation in the biomass of the pea increased significantly due to the absorption of plant available nitrogen by the weeds, however, the decrease of pea yield due to competition lead to a decrease of the symbiotic fixed N<sub>2</sub> amount per hectare for the pea (Corre-Hellou & Crozat 2005). In order to optimise the symbiotic N<sub>2</sub> fixation of the legumes in ecological agriculture, the competition by weeds therefore has to be reduced to a small extent through an efficient weed control in legumes.

Not only competition of weeds but also non-legume intercrops in general lead to a crop-related decrease of the symbiotic N<sub>2</sub> fixation activity of the legume despite frequently higher yield of the intercrop. So the cultivation of peas intercropped with oats (*Avena sativa*) at seed densities in the mixture of 67% (pea) respectively 33% (oats) of the sole crops lead to a higher grain crop activity of the mixtures in comparison to the monocrops, but also at the same time to a lower N<sub>2</sub> fixation activity and a negative N balance (Table 4). In order to optimize the symbiotic N<sub>2</sub> fixation activity in the mixtures of legumes and non-legumes, it is therefore recommended to strive for a crop proportion of legume of 80% to 90%. Thus, advantages of intercropping like higher yielding (Table 4) and improved weed suppression (Kimpel-Freund et al. 1998, Saucke & Ackermann 2006) can at the same time be strategically used in ecological agriculture. Peas and oats in the mixture furthermore use the soil-borne nitrogen more intense than oats and peas grown as sole crops (Table 4), so that

here nitrogen is prevented from eluviation and can be productively used for crop activity. Analyses about intercropping of peas and barley (*Hordeum vulgare*) have shown that the grain in the mixture strikes roots deeper due to competition and uses resources from the subsoil in a stronger way (Hauggaard-Nielsen et al. 2001).

**Table 4:** Grain yield, symbiotic N<sub>2</sub> fixation and N balance growing pea and oat as monocrops and intercrops in an organic field experiment (Schmidtke 1997)

	Year	Peas <sup>1)</sup>	Peas <sup>1)</sup> and Oats		Oats
Grain yield [t DM ha <sup>-1</sup> ]	1995	4.08	1.21	3.76	4.89
	1996	4.87	1.36	4.46	5.56
N in grain [kg N ha <sup>-1</sup> ]	1995	132.2	39.3	49.9	73.6
	1996	160.6	46.1	77.4	85.3
Soil N <sup>2)</sup>	1995	66.4	17.4	84.5	99.3
	1996	77.5	11.1	104.7	107.6
N <sub>2</sub> fixation <sup>2)3)</sup> [kg N ha <sup>-1</sup> ]	1995	103.9	44.9	-	-
	1996	125.3	52.8	-	-
Fixed N <sub>2</sub> in rhizodeposits <sup>4)</sup>	1995	13.4	5.8	-	-
	1996	16.2	6.8	-	-
N balance	1995	-14.9	-38.5		-73.6
	1996	-19.1	-63.9		-85.3

<sup>1)</sup> Mean of 4 pea varieties, <sup>2)</sup> in shoot and roots, <sup>3)</sup> estimated by <sup>15</sup>N natural abundance method (Shearer & Kohl 1986), <sup>4)</sup> according to the results from pot experiments listed in Table 2

In a few cases the symbiotic N<sub>2</sub> fixation activity of the legume can admittedly be increased by intercropping with a non-legume. This is the case when a twining legume type grows with an upright growing non-legume in the mixture. In a mixture of maize (*Zea mays*) and ricebean (*Vigna umbellata*) for example a slightly higher symbiotic N<sub>2</sub> fixation than in the sole crop of the legume could be detected (Rerkasem & Rerkasem 1988).

By use of cultivation in ridges in comparison to flat cultivation an increase of the yield and N<sub>2</sub> fixation until the anthesis could be achieved for faba bean (Vinther & Dahlmann-Hansen 2005). Cultivation in ridges a higher soil temperature, a quicker establishment of the nodules as well as a deeper rooting in comparison to the flat cultivation could be remarked, which promoted the initial development of the crop. Nevertheless, at the time of grain harvest of the faba bean as well as during analogical experiments with the soybean (*Glycine max*, Pikul et al. 2001), no significant effect on the crop development respectively the N<sub>2</sub> fixation activity of the crop could be noted any longer (Vinther & Dahlmann-Hansen 2005).

Requirement for the achievement of a higher yield of legumes and an effective symbiosis is a sufficient maintenance of the soil with plant available nutrients as well as a sufficiently high pH-level in the soil (Sadowsky 2005). The level of the symbiotic N<sub>2</sub> fixation is particularly strongly influenced by a lack of sulphur, molybdenum and iron, which are required for the development of the nitrogenase (Marschner 1995). An insufficient balance of nutrients showed above all a lack in phosphorus (CAL: 5 mg P kg<sup>-1</sup>) and potassium (CAL: 110 mg K kg<sup>-1</sup>, pH: 5.4, luvisol from loess) in long-lasting ecologically farmed soils, which strongly

constrained the N accumulation of red clover (Römer & Lehne 2004). Through supply of compost from organic household residues and rock phosphate yield as well as nutrient uptake of the red clover could be significantly increased (Table 5). Composts high in potassium, like compost from organic household residues therefore seem to be very suitable to meet the potassium needs of the legumes. Lack of potassium leads to changes of the relative growth of root, nodules and the shoot and in that way influences the yield formation and symbiotic N<sub>2</sub> fixation activity of legumes (Høgh-Jensen 2003).

**Table 5:** Dry matter yield and shoot P, K, and N contents of red clover per pot (sum of three cuts, Römer & Lehne 2004)

	Shoot DM [g]	P [mg]	K [mg]	N [mg]
Control	24	44	748	764
Rock phosphate <sup>1)</sup>	39	103	987	1250
Compost from organic household residues <sup>2)</sup>	45	116	1683	1460

<sup>1)</sup>Fertilisation per pot: 400 mg P, <sup>2)</sup>Fertilisation per pot: 400 mg P and 1400 mg K

Yield formation and symbiotic N<sub>2</sub> fixation activity of the legumes are dependent on the establishment of an efficient symbiosis between macro- (plant) and micro-symbiot (bacterium) at limited provision of plant available nitrate in the soil. Provided that efficient micro-symbiots are not or only in small amounts available in the soil, the N<sub>2</sub> fixation activity can be increased by an inoculation of the legume seeds or the soil (Hardason & Atkins 2003).

Water delimits the crop development and N<sub>2</sub> fixation activity of the legumes, especially if they have, similar to the pea, only a comparatively flat rooting depth (Reiter et al. 2002b). Some types of legumes, like for example the narrow-leaved lupine (*Lupinus angustifolius*) or the lucerne have proven to be very suitable for cultivation in arid environments, because they have an ability for deep rooting (Anderson et al. 1998). With this system and an adequate rooting of the soil they can adopt deep water reserves in the soil and survive dry phases with low crop losses. Moreover, within the range of legume genotypes exist, which can achieve higher performances in crop and symbiotic N<sub>2</sub> fixation under water limited circumstances (Sinclair et al. 2007).

In legume-based crop rotations it furthermore needs to be considered that an additional water usage with symbiotic N nutrition of the plant occurs, which under water-limited conditions can limit the crop and N<sub>2</sub> fixation activity. Recent investigations on the water use of pea have shown that symbiotically-grown plants had a significantly lower transpiration efficiency of 4.5 g dry matter L<sup>-1</sup> H<sub>2</sub>O compared to nitrate-fed pea which produced 5.5 g dry matter L<sup>-1</sup> H<sub>2</sub>O (Table 6). These results were achieved under glasshouse conditions. The transpiration efficiency of pea was measured in pots which were filled with coarse sand. The surface of the pots was covered by a membrane sheet to prevent water loss from the soil through evaporation.

A simple calculation using the results from this pot experiment underlines the importance of the nature of the N source (symbiotic or nitrate) on yield formation of legumes under water limited conditions. Pea crops, grown under comparable conditions to the glasshouse experiment and provided with 200 mm of plant-available water for transpiration, would be able to produce 11 t (total plant dry matter) ha<sup>-1</sup> if the plants were fully dependent on soil or fertilizer N. If peas were fully reliant on symbiotically fixed N<sub>2</sub>, a total plant dry matter yield of only 9 t ha<sup>-1</sup> should be attainable with 200 mm of water for transpirational water use. From the data on the transpiration efficiency of N accumulation (Table 6), it can be concluded that a pea crop under growing conditions comparable to the glasshouse experiment needs 204 mm plant-available water for transpiration to accumulate 200 kg N ha<sup>-1</sup> via symbiotic N<sub>2</sub> fixation and only 149 mm water to accumulate 200 kg N ha<sup>-1</sup> from soil or fertilizer-sourced N.

**Table 6:** Influence of symbiotic and nitrogen N source on total plant dry matter (DM), N yield and transpiration efficiency<sup>1)</sup> based on dry matter and on nitrogen accumulation in pea<sup>2)</sup> (Schmidtke 2006)

N source	Total plant <sup>2,3)</sup> dry matter (g pot <sup>-1</sup> )	Total plant <sup>2,3)</sup> N (g pot <sup>-1</sup> )	Transpiration efficiency of total plant DM accumulation (g DM L <sup>-1</sup> )	Transpiration efficiency of total plant N accumulation (mg N L <sup>-1</sup> )
Symbiotic	50.4	1.102	4.5	9.77
Nitrate	42.1	0.991	5.5	13.44
<i>P value</i>	0.1088	0.2943	0.0004	0.0001

<sup>1)</sup>transpirational water use is the cumulative transpiration during growth and transpiration efficiency is the ratio of total plant DM and transpirational water use; <sup>2)</sup>mean values of the two cultivars Grapis and Eiffel, no significant difference between cultivars and no significant interaction cultivar × N source was found; <sup>3)</sup>total plant biomass: shoot and roots

Plants need to open the stomata to take up CO<sub>2</sub> for assimilation. Simultaneously, water vapour is lost via the stomata during this opening (transpiration). If most of the assimilated CO<sub>2</sub> is stored as assimilates in the plant biomass the transpiration efficiency measured as a ratio of total plant DM and total transpirational water consumption will be high. On the other hand if the assimilates are used as an energy source for symbiotic N<sub>2</sub> fixation (respiration of assimilates), the transpiration efficiency decreases so that less plant dry matter is produced for one unit of transpirational water consumption. Therefore, due to the high respiratory costs of symbiotic N<sub>2</sub> fixation and its additional water consumption for symbiotic N<sub>2</sub> fixation, as can be seen from these results (Table 6), yield formation of legume crops will be more limited when the majority of plant N is derived from symbiotic N<sub>2</sub> fixation and plant-available water is a limiting factor in plant production.

The results of pot experiments in the green house could in the meantime be approved by field experiments. So the overall water usage for the development of one kilogram dry mass increased to ca. 103 litre (pea) respectively 76 litre (field bean) in comparison to crops supplied with mineral nitrogen. Under the water limited circumstances in the field, the higher water usage of the mainly symbiotic nourished legumes was related to a 20% low crop yield (Eisenreich & Schmidtke 2007). Therefore, a high meaning must be assigned to a sufficient water supply, e.g. through irrigation, as strategy for optimisation of the symbiotic N<sub>2</sub> fixation rate of the legumes under dry conditions.

## Reduction of plant available soil nitrogen

Legumes reduce the symbiotic N<sub>2</sub> fixation with increase of plant available nitrogen in the soil (Viosin et al. 2002). Therefore, legumes should not be fertilised with organic manures or compost which contain higher concentration of plant available nitrogen (e.g. slurry or liquid manure) respectively those whose organic nitrogen is quickly mineralised after the incorporation into the soil (e.g. horn clippings).

It is known from crop rotation experiments that the intensity of nitrogen fertilisation of the preceding crop can also have an influence on the N<sub>2</sub> fixation activity. During cultivation of soybean (*Glycine max*, Oberson et al. 2007), lucerne, faba bean and common vetch (*Vicia sativa*, Anthes 2005) in one crop rotation with long-lasting high N supply of preceding crops, significantly lower N<sub>2</sub> fixation activities of the legumes could be detected. Therefore, for optimizing the symbiotic N<sub>2</sub> fixation activities the legumes in the crop rotation should be integrated at one point in time when the nitrogen supply in the soil is very low. Consequently, it should be avoided to cultivate one legume after a legume catch crop or a legume main crop.

In order to reduce plant available nitrogen in the surface soil and subsoil before the cultivation of legumes, a non-legume cover crop can be cultivated. In contrary to the potato (*Solanum tuberosum*), cereals has been proven very suitable as preceding crop for legumes, because the cereals in general absorb high amounts of nitrogen out of the soil. In addition, the harvest residues of cereals often lead to an immobilisation of mineral nitrogen in the soil after incubation. Therefore, it was also tried to increase the symbiotic N<sub>2</sub> fixation activity of legumes through a targeted incubation of straw.

The incubation of higher amounts of straw in one of three experiments led to a significant increase of the symbiotic N<sub>2</sub> fixation activity of pea (Table 7). The incubated straw induced a temporary immobilisation of mineral nitrogen in the surface soil, which was mirrored in a lower soil derived nitrogen absorption by pea after straw manuring. However, barley reacted to the straw manuring with a considerable reduction of the soil-borne N absorption (Table 8).

**Table 7:** Influence of soil treatment with cereal straw on shoot N derived from soil and symbiotic N<sub>2</sub> fixation in pea and barley (Evans et al. 1997)

Soil treatment	Shoot N [kg N ha <sup>-1</sup> ]			
	Pea			Barley
Straw [t ha <sup>-1</sup> ]	Soil N	fixed N <sub>2</sub>	Total	Soil N
0.0	15.1	53.6	68.7	31.4
2.5	11.0	62.6	73.6	24.7
5.0	8.1	76.2	84.3	20.8
10.0	10.5	68.5	79.0	22.7

Evans et al. (1997) have concluded that under field conditions the benefit of soil-incorporated straw in increasing the N fixed by pea appears to be limited to situations with moderate mineral N concentrations in surface soil together with mineral N content in subsoil. Only a small part of this benefit appears to drive from immobilisation of soil nitrogen. Straw may increase fixed N by increasing crop growth, but too much straw seems likely to reduce crop N. It appears the amount of straw should not exceed 5 t ha<sup>-1</sup> (Evans et al. 1997).

By withdrawal of the intensity of tillage for the seed of legume, the nitrogen mineralisation in the soil can be reduced and therefore of plant available nitrogen in the soil can be decreased during the cultivation of legume. Since many years, undersowing of forage legumes in the cover crop is practiced very successfully in ecological agriculture. In this connection the legume is often sown without treatment of the soil (direct seeding), so that the legume catch crop has only a small amount of soil-borne nitrogen available due to the defaulted soil treatment and the N absorption of the cover crop. The nitrogen fixation activity of the legume is clearly activated through this.

The withdrawal of the intensity of tillage during the sowing of the grain legume proved to be effective for long-term reduced (Table 8) as well as for short-term varied intensity of tillage (Table 9). Even after 28 years of permanent reduced tillage activity the nitrogen fixation activity could be increased for the pea in comparison to the treatment with the plough, because reduced cultivation in spring leads to a deceleration of nitrogen mineralization in the soil in comparison to the treatment with the plough (Reiter et al. 2002b). In the course of the whole vegetation period it could however be recognised that for long-term reduced tillage similar high amounts of nitrogen in the soil became plant available as after the long-term land management with the plough. Therefore, for the red clover only small differences in the symbiotic N<sub>2</sub> fixation activity could be remarked (Table 8). However, in ecological agriculture, a long-term abandonment of plough use is very difficult because the growth and the reproduction of weeds during treatments without the plough are strongly supported. Therefore, a short-term withdrawal of tillage intensity in order to limit plant available nitrogen in the soil might possibly be assigned a higher meaning in ecological agriculture.

**Table 8:** Influence of long-term tillage systems (plow: CT, rotary harrow: MT) on grain and herbage yield, symbiotic N<sub>2</sub> fixation and N balance of pea and red clover (after Reiter et al. 2002b)

	Year	Peas		Red clover	
		CT	MT	CT	MT
Grain/herbage yield [t DM ha <sup>-1</sup> ]	1998	2.62	2.90	7.21	7.34
	1999	3.40	3.11	8.13	8.30
N in grain/in herbage [kg N ha <sup>-1</sup> ]	1998	66	82	208	216
	1999	99	81	223	211
Soil N <sup>1)</sup> [kg N ha <sup>-1</sup> ]	1998	66	48	122	100
	1999	63	28	125	88
N <sub>2</sub> fixation <sup>2)3)</sup> [kg N ha <sup>-1</sup> ]	1998	48	77	147	177
	1999	74	82	208	212
Fixed N <sub>2</sub> in rhizodeposits <sup>4)5)</sup>	1998	6	10	8	9
	1999	10	11	11	11
N balance	1998	-6	+5	-53	-30
	1999	-15	+11	-4	+12

<sup>1)</sup> in shoot and roots, <sup>2)</sup> estimated by large scale, low-level <sup>15</sup>N-application technique (Reiter et al. 2002a), <sup>3)</sup> according to the results for pea from pot experiments listed in Table 2, <sup>4)</sup> according to the results for red clover from pot experiments listed in Table 2

After rye grass (*Lolium perenne*) catch crop, the soil treatment was one-time varied before the sowing of pea and faba bean (Table 9). After a reduced treatment with the rotary harrow, pea and faba bean were sown in direct seeding into dying rye grass. Here direct seeding led to an increase of the crop yield and symbiotic N<sub>2</sub> fixation activity of the peas but not to a decrease of the N absorption from the soil (Table 9). For the faba bean no difference between the tillage treatments could be remarked. Direct seeding had contributed to more water being available in the soil for the crop development of the pea, but after the rye grass catch crop had not led to a limitation of N mineralization in the soil. In the conventional cultivation soybeans are cultivated since many years in direct seeding in South America (Hungria et al. 2005). The no-till agriculture here is carried out into a mulch layer of residues of the preceding crop.

**Table 9:** Influence of short-term tillage systems (rotary harrow: MT, zero tillage: ZT) on grain yield and symbiotic N<sub>2</sub> fixation of pea and faba bean in 2006 (Ernst & Schmidtke, unpublished)

	Pea		Faba bean	
	MT	ZT	MT	ZT
Grain yield [t DM ha <sup>-1</sup> ]	2.38	2.98	3.11	3.07
N in grain [kg N ha <sup>-1</sup> ]	72.8	91.4	137.7	139.6
Soil N <sup>1)</sup> [kg N ha <sup>-1</sup> ]	65.6	63.1	70.4	69.6
N <sub>2</sub> fixation <sup>1)2)</sup> [kg N ha <sup>-1</sup> ]	42.9	83.8	141.4	144.1

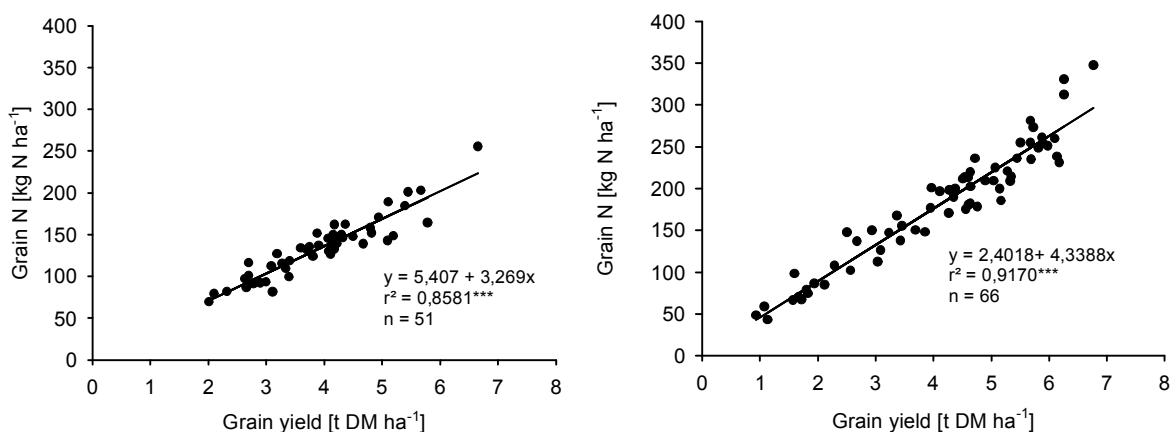
<sup>1)</sup> in shoot, <sup>2)</sup> estimated by large scale, low-level <sup>15</sup>N-application technique (Reiter et al. 2002a)

The effects of the sole withdrawal of tillage intensity on the increase of the symbiotic N<sub>2</sub> fixation of grain legumes is obviously not very strong (Table 8 and Table 9), so that a combination of strategies for the decrease of mineral nitrogen in the soil during legume growth needs to be aspired. This includes the cultivation of grain legume after cereals,

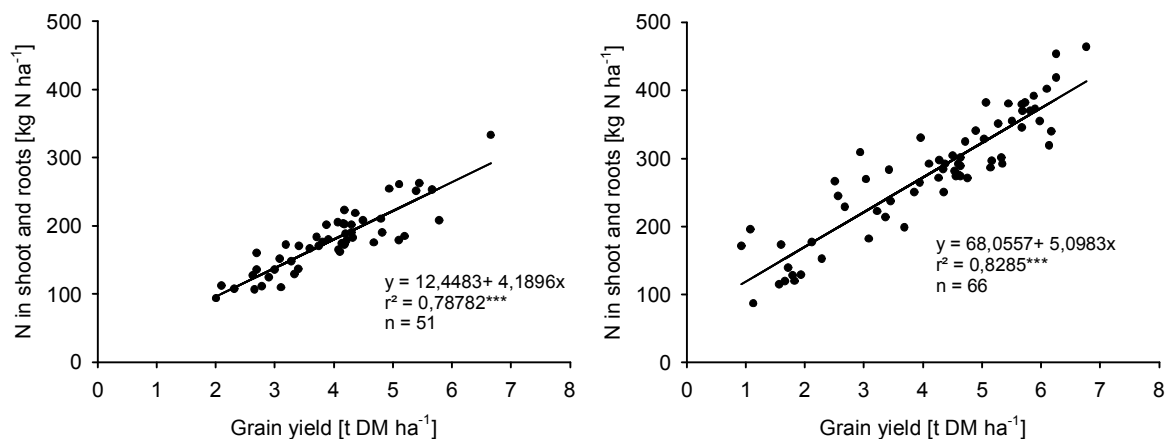
leaving the cereal straw on the agricultural area, the cultivation of a non-legume catch crop before the grain legume, whose biomass is only delayed decomposed and the reduction of soil management during the sowing of the grain legume.

#### 4 Calculations of the N-flows during the cultivation of legumes

A productive and at the same time environment-friendly crop production in ecological farming and gardening implies that the nitrogen supply can be calculated and purposeful controlled. In legume based crop rotations the calculation of the N<sub>2</sub> fixation and the N balance related to the cultivation of legumes is particularly difficult, because the level of the N<sub>2</sub> fixation activity is dependent on many factors. However, for some legumes it is possible now to calculate the nitrogen flows by means of the data available in practice (Jost & Schmidtke 2008). The adequate calculation programme is available from the internet for science, advisory services and practice in ecological agriculture ([www.isip2.de](http://www.isip2.de)). By means of data about grain production, soil quality, the level of occurring harvest losses, the degree of weed infestation of the crop as well as an estimation of the N availability in the soil through the preceding crop, this calculation programme assesses and displays the level of the symbiotic N<sub>2</sub> fixation activity of the legume and the related N balance online. For the development of the calculation programme, results of field experiments for N accumulation in shoot and roots of the legumes and the symbiotic N<sub>2</sub> fixation as well as pot experiments about the level of the N-rhizodeposition have been used. With the example of the pea and the faba bean the development of the calculation process should be explained in the following paragraph and important conclusions for the composition of cultivation systems in Organic Agriculture should be deflected.



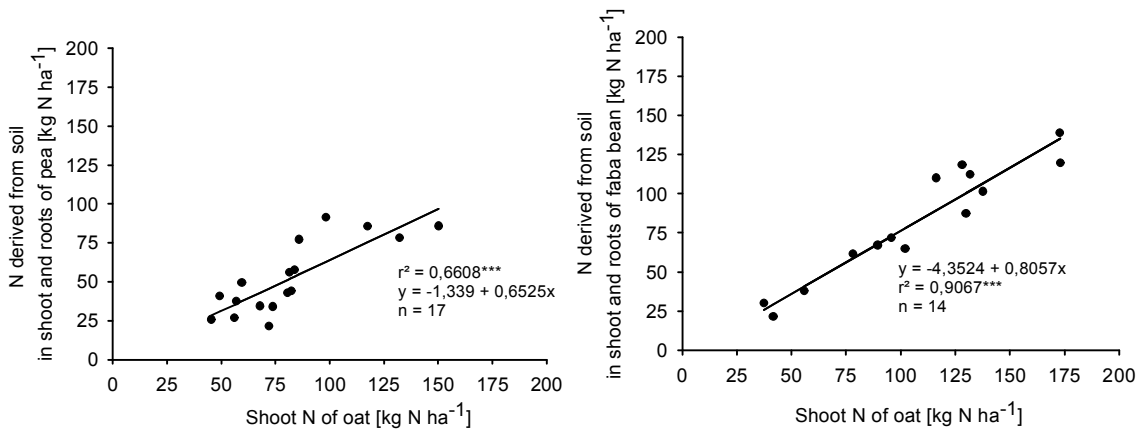
**Figure 2:** Influence of the grain yield on the N amount in grain for pea (left) and faba bean (right)



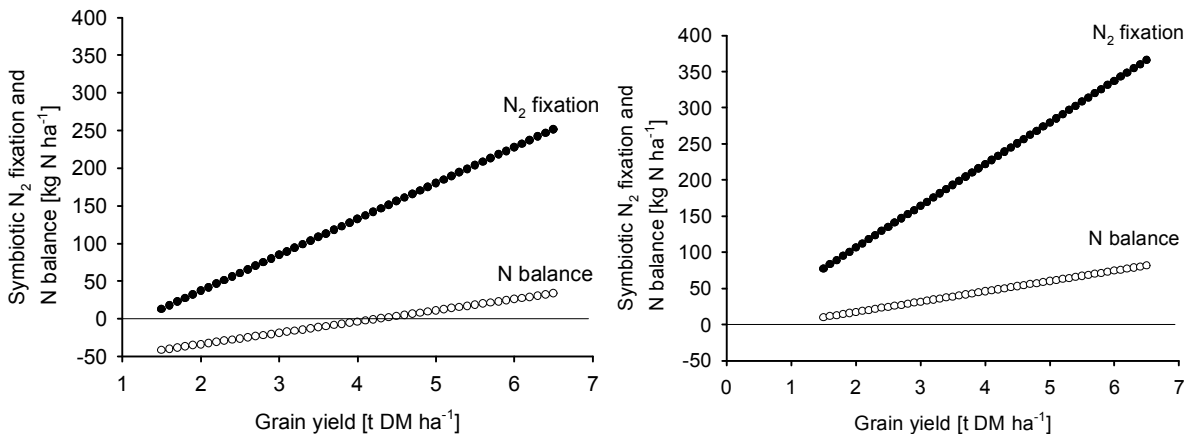
**Figure 3:** Influence of the grain yield on the N amount in shoot and roots for pea (left) and faba bean (right)

Information about the grain yield can help to calculate the N amount in grain (Figure 2) as well as the overall N amount in shoot and roots (Figure 3). The N amount absorbed from the soil by the grain legumes can directly be transferred through the N amount in the shoot into the growing oat nearby the grain legume (Figure 4). For this purpose the N amount of the grain legumes absorbed by the soil was investigated with the  $^{15}\text{N}$  natural abundance method independent of the total N absorption of the oat. Therefore, the soil-borne N amount in the biomass of the grain legume can be suggested from the N accumulation in the shoot of the oat (Figure 4). The results shown in Figures 2 to 4 include data of experiments by Gerling (1983), Busch (1987), Königs (1987), Dekhuijzen et al. (1984), Hauser (1987), Protzmann (1991), Aufhammer et al. (1994), Haynes et al. (1993), Göbel (1995), Schmidtke (1997), Kaul (1998), Schmidtke (2001), Reiter et al. (2002b), Jost (2003) and Anthes (2005). Per unit of grain yield the faba bean showed a higher N amount in grain compared to the pea (Figure 2) and also a higher N amount in shoot and roots (Figure 3). Furthermore, the faba bean used the nitrogen available in the soil – mirrored by the N amount in the shoot of the oat – in a stronger way than the pea (Figure 4). In consideration of the N rhizodeposition for the pea and faba bean (Table 2) it results a lower  $\text{N}_2$  fixation activity and lower N balance at the same grain yield and amount of plant-available N for the pea in comparison to the faba bean (Figure 5).

Therefore, for a grain yield on the level of  $2 \text{ t ha}^{-1}$  and an amount of plant-available soil of  $100 \text{ kg N ha}^{-1}$  the symbiotic  $\text{N}_2$  fixation activity of the pea is about  $36 \text{ kg N ha}^{-1}$ , and for the field bean respectively  $106 \text{ kg N ha}^{-1}$ . The N balance in this case adds up to  $-34 \text{ kg N ha}^{-1}$  (pea) respectively  $+17 \text{ kg N ha}^{-1}$  (faba bean). As long as the crops with the same amount of plant-available N in the soil each achieve a grain yield of  $5 \text{ t ha}^{-1}$ , the adequate values for the pea increase to 180 respectively  $+11 \text{ kg N ha}^{-1}$  and for the field bean to 279 respectively  $+60 \text{ kg N ha}^{-1}$  (Figure 5). The level of the grain yield therefore identifies with given amounts of plant available soil N the  $\text{N}_2$  fixation activity and the N balance very strongly. The comparison of the calculation results of pea and field bean underlines the meaning of the choice of legume type for the nitrogen supply in ecological agriculture. Provided that the same grain yield can be achieved with the cultivation of pea and faba bean at the site, cultivation of field bean is recommended, because a higher  $\text{N}_2$  fixation activity as well as a higher N balance is achieved during removal of the harvested grain (Figure 5).

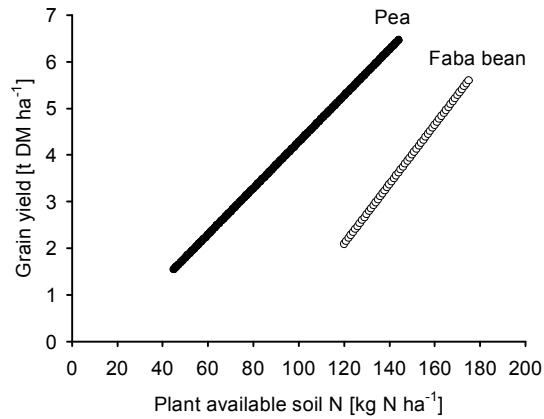


**Figure 4:** Influence of the N amount in the shoot of an oat growing close to the grain legume on the N absorption out of the soil by the pea (left) and the faba bean (right), N-absorption out of the soil for pea and faba bean has been investigated by the <sup>15</sup>N natural abundance method.



**Figure 5:** Influence of the grain yield on symbiotic N<sub>2</sub> fixation and N balance during the cultivation of the pea (left) and the faba bean (right), amount of plant-available soil N = 100 kg N ha<sup>-1</sup>

In Figure 6 the straight lines show which level of grain yield has to be achieved for pea and faba bean in order to reach an N balance of 0 kg N ha<sup>-1</sup>. In this way, already starting from a provision of plant available nitrogen in the soil of 45 kg N ha<sup>-1</sup> for the pea a grain yield of 1.54 t ha<sup>-1</sup> is required in order to reach an N balance of 0 kg N ha<sup>-1</sup>. However, the grain yield has to be at least 5.26 t ha<sup>-1</sup>, in order to guarantee a balanced N situation with 120 kg N ha<sup>-1</sup> of plant available nitrogen in the soil of (Figure 6). Growing faba bean and the amount of plant available nitrogen in the soil is less than 120 kg N ha<sup>-1</sup>, the N balance is even for low grain crops still positive. But if plant available nitrogen in the soil is at 120 kg N ha<sup>-1</sup>, then a grain yield of at least 2.09 t ha<sup>-1</sup> is required here for the achievement of a positive N balance. The level of grain yield required for the balanced N situation increases for the faba bean to 5.28 t ha<sup>-1</sup>, as long as the amount of plant available nitrogen in the soil accounts for 170 kg N ha<sup>-1</sup> (Figure 6). The decrease of the plant available in the soil is therefore significantly more important during the cultivation of the pea for grain usage than during cultivation of the faba bean, in order to ensure the N supply in crop rotations of ecological agriculture.



**Figure 6:** Required grain yield for pea and faba bean for the achievement of a N balance of 0 kg N ha<sup>-1</sup> as function of the amount of plant-available N in the soil

It will be a research task to develop adequate site-related calculation processes for the estimation of the symbiotic N<sub>2</sub> fixation activity and the N balance also for further types of legumes, such as for example the soybean, in order to more strategically plan and more efficiently ensure the N supply through an optimisation of the symbiotic N<sub>2</sub> fixation activity of cultivated legumes.

## 5 Conclusions

By means of the analysis results and calculation methods presented here it could be shown that many possibilities exist in Organic Agriculture to optimise the symbiotic N<sub>2</sub> fixation activity of cultivated legumes through agricultural management. Here stronger impacts on the level of the symbiotic N<sub>2</sub> fixation activity through the choice of a site-related legume type and procedures to increase the yield of legumes have to be expected. By means of a decrease in plant available nitrogen in the soil during the cultivation of legumes the symbiotic N<sub>2</sub> fixation activity of the legumes can be additionally increased. However, the agricultural procedures suitable for this are more difficult to use in ecological agriculture practice and are related to a lower impact on the level of symbiotic N<sub>2</sub> fixation of the legumes.

## 6 References

- Anderson G.C.; Fillery, I.R.P.; Dunin, F.X.; Dolling, P.J. & Asseng, S. (1998) Nitrogen and water flows under pasture-wheat and lupin-wheat rotations in deep sands in Western Australia. 2. Drainage and nitrate leaching. *Australian Journal of Agricultural Research* **49**, 345-361
- Anthes, J. (2005) Beitrag von Ackerbohne (*Vicia faba* L.), Luzerne (*Medicago sativa* L.) und Saatwicke (*Vicia sativa* L.) zur Selbstregelung der N-Zufuhr in leguminosenbasierten Fruchtfolgen. PhD-Thesis University of Goettingen, Germany
- Aufhammer, W.; Fiegenbaum, A. & Kübler, E. (1994) Zur Problematik der Stickstoffrückstände von Ackerbohnen (*Vicia faba* L.). Teil 1: Stickstoffakkumulation und Stickstoffrückstände von Ackerbohnen. *Die Bodenkultur* **45**, 239-251
- Busch, M. (1987) Die symbiotische Stickstoff-Bindung von Ackerbohnen und Erbsen in einem Feldversuch im Jahr 1986. Diploma-Thesis Institute of Agronomy and Plant Breeding University of Goettingen, Germany
- Corre-Hellou, G. & Crozat, Y. (2005) N<sub>2</sub> fixation and N supply in organic pea (*Pisum sativum* L.) cropping systems as affected by weeds and pea weevil (*Sitona lineatus* L.). *European Journal of Agronomy* **22**, 449-458
- Dekhuijzen, H.M. & Verkerke, D.R. (1984) Uptake, distribution and redistribution of <sup>15</sup>Nitrogen by *Vicia faba* under field conditions. *Field Crops Research* **8**, 93-104
- Eisenreich, F. & Schmidtke, K. (2007) Untersuchungen zur Wassernutzungseffizienz von Körnerleguminosen. *Mitteilungen der Gesellschaft für Pflanzenbauwissenschaften* **19**, 304-305
- Evans, J., Fettell, N.A.; O'Connor, G.E.; Carpenter, D.J.; Chalk, P.M. (1997) Effect of soil treatment with cereal straw and method of crop establishment on field pea (*Pisum sativum* L.) N<sub>2</sub> fixation. *Biology and Fertility of Soils* **24**, 87-95
- Frame, J.; Charlton, J.F.L.; Laidlaw, A.S. (1998) *Temperate Forage Legumes*. CAB International, New York
- Gerling, H. (1983) Untersuchungen über den Nitratfluß in einer Para-Braunerde aus Löß und über die Stickstoffaufnahme durch Ackerbohnen und Hafer. Diploma-Thesis Institute of Agronomy and Plant Breeding University of Goettingen, Germany
- Göbel, J. (1995) Zur Produktivität von Winterraps- und Wintergerstenbeständen, erstellt als Untersaaten in Ackerbohnenbeständen. PhD-Thesis University of Hohenheim, Germany.
- Hardason, G. & Atkins, C. (2003). Optimising biological N<sub>2</sub> fixation by legumes in farming systems. *Plant and Soil* **252**, 41-54
- Hauggaard-Nielsen, H.; Ambus, P. & Jensen, E.S. (2001) Temporal and spatial distribution of roots and competition for nitrogen in pea-barley intercrops – a field study employing <sup>32</sup>P technique. *Plant and Soil* **236**, 63-74
- Hauser, S. (1987) Schätzung der symbiotisch fixierten Stickstoffmenge von Ackerbohnen (*Vicia faba* L.) mit erweiterten Differenzmethoden. PhD-Thesis University of Goettingen, Germany
- Haynes, R.J.; Martin, R.J. & Goh, K.M. (1993) Nitrogen fixation, accumulation of soil nitrogen and nitrogen balance for some field-grown legume crops. *Field Crops Research* **35**, 85-92
- Høgh-Jensen, H. & Schjoerring, J.K. (2001) Rhizodeposition of nitrogen by red clover, white clover and ryegrass leys. *Soil Biology & Biochemistry* **33**, 439-448
- Høgh-Jensen, H. (2003) The effect of potassium deficiency on growth and N<sub>2</sub>-fixation in *Trifolium repens*. *Physiologia Plantarum* **119**, 440-449
- Hungria, M.; Franchini, J.C.; Campo, R.J. & Graham, P.H. (2005) The importance of nitrogen fixation to soybean cropping in South America. In: Werner, D. & Newton, W.E. (eds) *Nitrogen Fixation in Agriculture, Forestry, Ecology, and the Environment*. Springer, Dordrecht, 25-42
- Jensen, E.S. (1996a) Grain yield, symbiotic N<sub>2</sub> fixation and interspecific competition for inorganic N in pea-barley intercrops. *Plant and Soil* **182**, 25-38
- Jensen, E.S. (1996b) Rhizodeposition of N by pea and barley and its effect on soil N dynamics. *Soil Biology & Biochemistry* **28**, 65-71

- Jost, B. & Schmidtke, K. (2008) Kalkulation der symbiotischen N<sub>2</sub>-Fixierleistung und der N-Flächenbilanz beim Anbau von Leguminosen im ökologischen Landbau. Abschlussbericht des Vorhabens (AZ 23181), gefördert von der Deutschen Bundesstiftung Umwelt, Germany, 1-72
- Jost, B. (2003) Untersuchungen und Kalkulationstabellen zur Schätzung der N<sub>2</sub>-Fixierleistung und der N-Flächenbilanz beim Anbau von *Lupinus albus* und *Lupinus luteus* in Reinsaat und von *Vicia faba* und *Pisum sativum* in Reinsaat und im Gemenge mit *Avena sativa*. PhD-Thesis University of Goettingen, Germany
- Jung, R. (2003) Stickstoff-Fixierleistung von Luzerne (*Medicago sativa* L.), Rotklee (*Trifolium pratense* L.) und Persischem Klee (*Trifolium resupinatum* L.) in Reinsaat und Gemenge mit Poaceen. PhD-Thesis University of Goettingen, Germany
- Kaul, H.-P. (1998) Analyse und Modellierung des Vorfrucht-Nachfrucht-Systems unter besonderer Berücksichtigung der Stickstoffflüsse nach dem Anbau öl- und eiweißreicher Körnerfruchtarten. Thesis University of Hohenheim, Germany
- Khan, D.F.; Peoples, M.B.; Chalk, P.M. & Herridge, D.F. (2002) Quantifying below-ground nitrogen of legumes. 2. A comparison of <sup>15</sup>N and non-isotopic methods. *Plant and Soil* **239**, 277-289
- Kimpel-Freund, H.; Schmidtke, K. & R. Rauber, R. (1998) Einfluß von Erbsen (*Pisum sativum* L.) mit unterschiedlichen morphologischen Merkmalen in Reinsaat und Gemenge mit Hafer (*Avena sativa* L.) auf die Konkurrenz gegenüber Unkräutern. *Pflanzenbauwissenschaften* **2**, 25-36
- Königs, I. (1987) Durchwurzelung des Bodens von Hafer, Erbsen und Ackerbohnen und im Boden verbleibende Nitratmengen – Beobachtungen an einem Feldversuch 1986. Diploma-Thesis Institute of Agronomy and Plant Breeding University of Goettingen
- Köpke, U. (1996) Symbiotische Stickstoff-Fixierung und Vorfruchtwirkung von Ackerbohnen (*Vicia faba* L.). Schriftenreihe Institut für Organischen Landbau **6**, 1-113. Verlag Dr. Köster, Berlin, Germany
- Mahieu, S.; Fustec, J.; Faure, M.-L.; Corre-Hellou, G. & Crozat, Y. (2007) Comparison of two <sup>15</sup>N labelling methods for assessing nitrogen rhizodeposition of pea. *Plant and Soil* **295**, 193-205
- Marschner, H. (1995) Mineral nutrition of higher plants. Academic Press, London
- Mayer, J.; Buegger, F.; Jensen, E.S.; Schloter, M. & Hess, J. (2003) Estimating N rhizodeposition of grain legumes using a <sup>15</sup>N in situ stem labelling method. *Soil Biology & Biochemistry* **35**, 21-28
- McAuliffe, C.; Chamblee, D.S.; Uribe-Arango, H. & Woodhouse, W.W. (1958) Influence of inorganic nitrogen on nitrogen fixation by legumes as revealed by <sup>15</sup>N. *Agronomy Journal* **50**, 334-337
- McNeill, A.M.; Zhu, C. & Fillery, I.R.P. (1998) A new approach to quantifying the N benefit from pasture legumes to succeeding wheat. *Australian Journal of Agricultural Research* **49**, 427-36
- Oberson, A.; Nanzer, S.; Bosshard, C.; Dubois, D.; Mäder, P. & Frossard, E. (2007) Symbiotic N<sub>2</sub> fixation by soybean in organic and conventional cropping systems estimated by <sup>15</sup>N dilution and <sup>15</sup>N natural abundance. *Plant and Soil* **290**, 69-83
- Peoples, M.B.; Faizah, A.W.; Rerkasem, B. & Herridge, D.F. (eds.) (1989) Methods for evaluating nitrogen fixation by nodulated legumes in the field. ACIAR (Australian Centre for International Agricultural Research, Canberra) Monograph No. 11
- Pikul, J.L.; Carpenter-Boggs, L.; Vigil, M.; Schumacher, T.E.; Lindstrom, M.J. & Riedell, W.E. (2001) Crop yield and soil condition under ridge and chisel-plow tillage in the northern Corn Belt. *Soil and Tillage Research* **60**, 21-33
- Protzmann, M. (1991) Zur Vorfrucht- und Stickstoffwirkung von Leguminosen unter besonderer Berücksichtigung der Lupinen (*Lupinus albus* L. und *Lupinus luteus* L.). PhD-Thesis University of Gießen, Germany
- Reiter, K., Schmidtke, K., Rauber, R. (2002b) The influence of long-term tillage systems on symbiotic N<sub>2</sub> fixation of pea (*Pisum sativum* L.) and red clover (*Trifolium pratense* L.). *Plant and Soil* **238**, 41-55

- Reiter, K.; Schmidtke, K. & Rauber, R. (2002a) Estimation of symbiotic N<sub>2</sub> fixation by a low-level, large-scale <sup>15</sup>N-application technique. *Soil Biology & Biochemistry* **34**, 303-314
- Rerkasem, K., & Rerkasem, B. (1988) Yields and nitrogen nutrition of intercropped maize and ricebean (*Vigna umbellata* [Thumb.] Ohwi and Ohashi). *Plant and Soil* **108**, 151-162
- Römer, W. & Lehne, P. (2004) Vernachlässigte Phosphor- und Kaliumdüngung im ökologischen Landbau senkt die biologische Stickstofffixierung bei Rotklee und den Kornertrag bei nachfolgendem Hafer. *Journal of Plant Nutrition and Soil Science* **167**, 106-113
- Russell, C.A. & Fillery, I.R.P. (1996). *In situ* <sup>15</sup>N labelling of lupin below-ground biomass. *Australian Journal of Agricultural Research* **47**, 1035-1046
- Sadowsky, M.J. (2005) Soil stress factors influencing symbiotic nitrogen fixation. In: Werner, D. & Newton, W.E. (eds) *Nitrogen Fixation in Agriculture, Forestry, Ecology, and the Environment*. Springer, Dordrecht, 89-112
- Saucke, H. & Ackermann, K. (2006) Weed suppression in mixed cropped grain peas and false flax (*Camelina sativa*). *Weed Research* **46**, 453-461
- Sawatsky, N. & Soper, R.J. (1991) A quantitative measurement of the nitrogen loss from the root system of field peas (*Pisum arvense* L.) grown in the soil. *Soil Biology & Biochemistry* **23**, 255-259
- Schmidtke, K. (1997) Stickstoff-Fixierleistung und N-Flächenbilanz beim Anbau von Erbsen (*Pisum sativum* L.) unterschiedlichen Wuchstyps in Reinsaat und Gemengesaat mit Hafer (*Avena sativa* L.). *Mitteilungen der Gesellschaft Pflanzenbauwissenschaften* **10**, 63-64
- Schmidtke, K., (2001) Umweltgerechter Anbau von Leguminosen. Entwicklung und Anwendung eines Verfahrens zur Quantifizierung der N-Flächenbilanz. Abschlussbericht des Vorhabens AZ 07312, gefördert von der Deutschen Bundesstiftung Umwelt, Germany, 1-234
- Schmidtke, K. (2005a) How to calculate nitrogen rhizodeposition: a case study in estimating N rhizodeposition in the pea (*Pisum sativum* L.) and grasspea (*Lathyrus sativus* L.) using a continuous <sup>15</sup>N labelling split-root technique. *Soil Biology & Biochemistry* **37**, 1893-1897
- Schmidtke, K. (2005b) A model to predict the accuracy of measurements of legume N rhizodeposition using a split-root technique. *Soil Biology & Biochemistry* **37**, 829-836
- Schmidtke, K. (2005c) N-Rhizodeposition bei Leguminosen: Messgenauigkeit, Modellierung und Bedeutung für den Pflanzenbau. *Mitteilungen der Gesellschaft für Pflanzenbauwissenschaften* **17**, 387-388.
- Schmidtke, K. (2006) Effect of grain legumes on water-use efficiency in crop rotations. In: Grain legumes and the environment: how to assess benefits and impacts? European Association for grain legume research AEP (ed.), AEP, Paris, France, 61-66
- Shearer, G. & Kohl, D.H. (1986) N<sub>2</sub> fixation in field settings: estimation based on natural <sup>15</sup>N abundance. *Australian Journal of Plant Physiology*. **13**, 699-756
- Sinclair, T. R.; Purcell, L.C.; King, A.A.; Sneller, C.H.; Chen, P. & Vadez, V. (2007) Drought tolerance and yield increase of soybean resulting from improved symbiotic N<sub>2</sub> fixation. *Field Crops Research* **101**, 67-71
- Vinther, F.P. & Dahlmann-Hansen, L., 2005. Effects of ridging on crop performance and symbiotic N<sub>2</sub> fixation of fababeans (*Vicia faba* L.). *Soil Use and Management* **21**, 205-211
- Voisin, A.S.; Salon, C.; Munier-Jolain, N.G. & Ney, B. (2002) Quantitative effects of soil nitrate, growth potential and phenology on symbiotic nitrogen fixation of pea (*Pisum sativum* L.). *Plant and Soil* **243**, 31-42
- Wichern, F.; Mayer, J.; Joergensen, R. & Müller, T. (2007) Rhizodeposition of C and N in peas and oats after <sup>13</sup>C-<sup>15</sup>N double labelling under field conditions. *Soil Biology & Biochemistry* **39**, 2527-2537
- Wichmann, S. (2004) Ertragsleistung, Futterqualitätsentwicklung, N<sub>2</sub>-Fixierungsleistung und Vorfruchtwirkung von verschiedenen Körnerleguminosenarten in Reinsaat und im Gemenge mit Getreide. PhD-Thesis University of Kiel, Germany