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ORIGINAL PAPER

Symbiotic N_2 fixation by soybean in organic and conventional cropping systems estimated by ^{15}N dilution and ^{15}N natural abundance

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Abstract Nitrogen (N) is often the most limiting nutrient in organic cropping systems. N_2 fixing crops present an important option to improve N supply and to maintain soil fertility. In a field experiment, we investigated whether the lower N fertilization level and higher soil microbial activity in organic than conventional systems affected symbiotic N_2 fixation by soybean (*Glycine max*, var. Maple Arrow) growing in 2004 in plots that were since 1978 under the following systems: biodynamic (DYN); bio-organic (ORG); conventional with organic and mineral fertilizers (CON); CON with exclusively mineral fertilizers (MIN); non-fertilized control (NON). We estimated the percentage of legume N derived from the atmosphere (%Ndfa) by the natural abundance (NA) method. For ORG and MIN we additionally applied the enriched ^{15}N isotope dilution method (ID) based on residual mineral and organic ^{15}N labeled fertilizers that were applied in 2003 in microplots installed in ORG and MIN plots. These different enrichment treatments resulted in equal %Ndfa values. The %Ndfa obtained by NA for ORG and MIN was confirmed by the ID method, with similar variation. However, as plant growth was restricted by the microplot frames the NA technique provided more accurate estimates of the quantities of symbiotically fixed N_2 (Nfix). At maturity of soybean the %Ndfa ranged from 24 to 54%. It decreased in the order ORG > CON > DYN > NON > MIN, with significantly lowest value for MIN. Corresponding Nfix in above ground plant material ranged from 15 to 26 g N m^{-2}, with a decreasing trend in the order DYN = ORG > CON > MIN > NON. For all treatments, the N withdrawal by harvested grains was greater than Nfix. This shows that at the low to medium %Ndfa, soybeans did not improve the N supply to any system but removed significant amounts of soil N. High-soil N mineralization and/or low-soil P availability may have limited symbiotic N_2 fixation.

Keywords ^{15}N Isotope dilution · ^{15}N Natural abundance · Organic farming · Soybean · Symbiotic N_2 fixation · Reference plants

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Introduction

Nitrogen (N) is often the most limiting nutrient in organic cropping systems where no synthetic mineral N fertilizers are applied and where total N inputs are usually lower than in conventional systems (Hansen et al. 2000). Therefore, N_2 fixing crops present an important option to improve N supply and to maintain soil fertility (Stockdale et al. 2001). Organic farms have on average more legumes in rotation (Kirchmann and Bergstrom 2001), and estimates on N_2 fixation from the atmosphere at the farm level are greater for organic than conventional systems (Hansen et al. 2000).

However, to our knowledge comparisons of the N input into the soil–plant-system by symbiotic N_2 fixation of legume crops under organic versus conventional cropping systems do not exist. In a long-term field experiment near Basel, Switzerland, organic and conventional cropping systems are being compared since 1978 (Besson and Niggli 1991; Mäder et al. 2002). Starting from identical soil characteristics, the soils of the different treatments now differ in biological, chemical, and physical soil properties (Mäder et al. 2002). Particularly, soils that were for a long-time under organic cropping have a greater microbial biomass and activity than conventionally managed soils. Together with the lower total and mineral N inputs (Langmeier et al. 2002), this might affect symbiotic N_2 fixation from the atmosphere.

In 2004, we made use of soybean grown on the differently managed plots of the long-term field experiment to investigate the percentage of legume N derived from the atmosphere (%Ndfa) and the quantity of N_2 fixed (Nfix) through symbiotic N_2 fixation from the atmosphere. We applied the enriched ^{15}N isotope dilution (ID) and the natural abundance (NA) technique (Unkovich and Pate 2000). In both methods, the %Ndfa is obtained from the comparison of the isotopic composition of the fixing legume with that of a non-fixing reference plant. While the available soil N pool is being enriched with ^{15}N in the ID technique (Danso et al. 1993), the NA method relies on the slight natural enrichment of ^{15}N that is observed in many agricultural soils relative to atmospheric N_2 (Giller 2001; Gathumbi et al. 2002). The ID technique has been used frequently for estimating N_2 fixation by legume crops (McNeill et al. 1998). Its difficulty to establish a stable ^{15}N enrichment of soil mineral N in space and time has been alleviated by the use of residual ^{15}N labeled mineral fertilizers (Vinther and Jensen 2000). Additionally the use of slow release forms of ^{15}N labeled fertilizers has been proposed (Danso et al. 1993; McNeill et al. 1998). With advances in mass spectrometry the NA method is increasingly being used (Högberg 1997). Its principal advantage when compared to the ID method is that it requires no addition of N. This makes it applicable also to experimental settings that are either devoid of any N fertilization or of mineral N fertilizers, e.g., organic farming trials, and at larger scale where the use of ^{15}N labeled fertilizers would be too expensive.

The selection of appropriate reference plants that fix no N_2 from the atmosphere but that have temporal and spatial uptake patterns for mineral soil N similar to the fixing legume crop, is critical for both methods (Danso et al. 1993; Giller 2001). Non-fixing reference mono- or dicotyledonous crops (McNeill et al. 1998; Reiter et al. 2002), non-nodulating mutants of the legume (Okito et al. 2004) or non-legume weeds (Schwenke et al. 1998) have been used. The NA method additionally requires the isotopic composition of the legume grown exclusively on atmospheric N_2 (B-value) because of isotopic fractionation during N_2 fixation (Okito et al. 2004).

We used a microplot study to which ^{15}N labeled sheep feces, sheep urine, and mineral N, respectively, had been applied the year previous to soybean planting. This setting was used to apply the ID method using different residual enrichment treatments (EnrichTr). Because the microplots had been established only in two of totally five systems that we were going to study, we additionally applied the NA technique to all of them.

To test the ID and the NA method for estimating %Ndfa and Nfix in organically and conventionally managed plots we: (1) evaluate the isotopic composition of soil N, of N in reference plants which were weeds that grew nearby the sampled soybean plants, and of

soybean N; (2) present *B*-values of soybean obtained with inoculum from differently managed plots; (3) compare the %Ndfa obtained with different residual mineral and organic ^{15}N EnrichTrs; (4) compare %Ndfa and Nfix obtained by the ID and NA technique. (5) At the end we discuss the significance of symbiotic N_2 fixation in the studied systems.

Materials and methods

Field experiment, cropping systems, and soybean cropping

Symbiotic N_2 fixation from the atmosphere by soybean was investigated in a long-term field experiment in Therwil, near Basel (Switzerland). In this field trial, organic and conventional cropping systems are being compared since 1978 on a loamy-silt (10% sand, 73% silt, 17% clay) Haplic Luvisol developed on loess in a temperate climate (Siegrist et al. 1998). The concept and experimental design of the field trial, including the detailed description of the management practices, were presented by Besson and Niggli (1991) and Mäder et al. (2002). We included the bio-dynamic (DYN), bio-organic (ORG), conventional (CON), conventional-mineral (MIN) cropping systems, and a non-fertilized control (NON). The systems called CON nowadays fulfill the Swiss guidelines of integrated production (KIP 1999), which has become almost area-wide agricultural practice in Switzerland. The cropping systems differ in fertilization and plant protection strategy (Table 1). Average N inputs by fertilizers, N balances calculated as difference between N input through fertilizers minus N export by harvested products, and selected soil properties are shown in Tables 1 and 2, respectively. All cropping systems are replicated four times in the field trial.

The forth crop rotation period (1999–2005) included winter wheat, 2 years of grass-clover meadow, potatoes, winter wheat, soybean, and maize. Between winter wheat and soybean, a green manure mixture from which mainly *Phacelia tanacetifolia* grew was sown. Decay of this plant material had started during winter before it was ploughed into the soil by mid April before land preparation for soybean. Plots of all systems were sown on April 30, 2004 by inoculated seeds of the soybean (*Glycine max*., var. Maple Arrow). The inoculum used was *Rhizobium japonicum* strain G49. No N was applied to soybean of any cropping system. Only CON and MIN received 30 kg P/ha in the form of triple superphosphate and 250 (CON) or 125 (MIN) kg K/ha in the form of potassium sulfate with magnesia previous to sowing.

Enriched ^{15}N isotope dilution method

Microplot study

The ID method was applied using microplots installed in all four replicate plots (5 m width × 20 m length) of cropping systems ORG and MIN. The exact design of the microplot study is described in Bosshard (2007, in preparation). Briefly, the microplots are frames of 33 cm length, 14 cm width, and 23 cm height that were driven into the soil to 18 cm depth. To these microplots, ^{15}N labeled fertilizers had been applied in March 2003 to winter wheat. The ^{15}N labeled fertilizer components (EnrichTrs) either were sheep feces, sheep urine or mineral N (Table 3). In 2004, we made use of the residual labeled fertilizers remaining in the soil that permitted totally three estimates of symbiotic N_2 fixation per each of the four replicates of ORG and MIN, respectively. As incubation and plant studies show that ^{15}N labeled sheep manure is a slow release fertilizer (Sorensen et al. 1994; Thomsen et al. 2003), this setting gave rise to the comparison of residual mineral and a slow release EnrichTr.

With a surface area of 0.0462 m^2, a microplot contained two soybean plants. Each microplot was repeated twice per plot, which enabled two sampling dates, one on August 12 at late flowering/early pod filling (thereafter denoted late flowering) and one on September 22 at the maturity of the soybean plants. Senescent leaves that fell off the plants before maturity were

Table 1 Fertilization and plant protection in the organic and conventional cropping systems and in the unfertilized control of the long-term field experiment, with average N input and N balances for 26 years (1978–2003)

Measures	Cropping system				
	Bio-dynamic (DYN)	Bio-organic (ORG)	Conventional (CON)	Conventional-mineral (MIN)	Unfertilized control (NON)
Fertilization					
Type and level	Aerobically composted farmyard manure (FYM) and slurry (1.2/1.4 LU/ha/year)[a]	Slightly aerobically rotted FYM and slurry (1.2/1.4 LU/ha/year)[a]	Stacked FYM and slurry (1.2/1.4 LU/ha/year)[a] and mineral fertilizer according to official guidelines[b]	Exclusively mineral fertilizer according to official guidelines[b,c]	Non-fertilized
Total N input (kg/ha/year)	104	110	158	128	0
Mineral N input (kg/ha/year)	34	38	101	128	0
N balance (kg/ha/year)[d]	–98	–93	–87	–98	–141
Plant protection					
Weed control	Mechanical	Mechanical	Mechanical and herbicides	Mechanical and herbicides	Mechanical
Disease control	Indirect methods	Indirect methods, until 1991 copper	Fungicides (thresholds)	Fungicides (thresholds)	Indirect methods
Insect control	Plant extracts, biocontrol	Plant extracts, biocontrol	Insecticides (thresholds)	Insecticides (thresholds)	Plant extracts, biocontrol
Special treatments	Biodynamic preparations	None	Plant growth regulators	Plant growth regulators	Biodynamic preparations

[a] Increase from 1.2 to 1.4 livestock units (LU)/ha/year at the beginning of the third crop rotation period (1992–1998)

[b] Before 1992 the applied levels were 1.2 times the recommended fertilizer amounts (Besson and Niggli 1991)

[c] MIN was from 1978 to 1985 non-fertilized

[d] Difference between total N input by fertilizers and N output by products removed at harvest

Table 2 Properties of the soils (0–18 cm) of the different cropping systems (*CropSys*)

CropSys[a]	Soil properties							
	pH[b]	Total C[c] (g/kg)	Total N[c] (g/kg)	Mineral N[d] (mg/kg)	Microbial C[e] (mg/kg)	Microbial N[e] (mg/kg)	P–CO_2[f] (mg/kg)	K–CO_2[f] (mg/kg)
DYN	6.3 b	15.1	1.61	nd	127 c	26.0 c	0.99 b	7.3 b
ORG	6.4 b	13.1	1.48	8.6	116 bc	24.9 bc	0.99 b	8.3 b
CON	6.4 b	13.0	1.41	nd	95 ab	17.0 ab	1.46 c	14.1 c
MIN	6.3 b	13.3	1.43	5.8	94 ab	16.8 ab	1.16 bc	8.1 b
NON	6.1 a	11.9	1.30	nd	79 a	14.9 a	0.26 a	2.5 a
SEM	0.06	1.0	0.11	0.93	8.5	2.8	0.11	0.75
Anova								
Soure of variation								
CropSys	**	ns	ns	ns	**	*	***	***

Soils were sampled in September 2004 after the harvest of soybean

*, **, *** significant at 0.05, 0.01, and 0.001 probability level, respectively, *ns* Not significant; means followed by the same letter are not significant different (Fisher's LSD, $p < 0.05$)

[a] For cropping systems see Table 1

[b] pH determined in water

[c] Europa Scientific Roboprep CN coupled to a Tracermass mass spectrometer (Europa Scientific, England)

[d] Determined using the KCl-extraction method of Davidson et al. (1991)

[e] Determined using the fumigation-extraction method of Vance et al. (1987), no conversion factors applied

[f] Determined using CO_2-saturated water extraction of Dirks and Scheffer (1930)

collected. Plants were eradicated with the help of a small spade to loosen the soil such that the roots down to about 10 cm depth could be removed. Roots, especially fine roots, were not sampled quantitatively, but at the first sampling the nodulated root parts could be recovered. At maturity, nodules had died off and the harvested root parts were lignified, with only few fine roots present.

As reference plants, we used two non-N_2 fixing weeds growing in the microplots. The weeds were grasses or herbs typical for the weed flora of soybean in ORG or MIN, respectively, with species composition similar to the weeds sampled for the NA technique (Table 4). The shoots of the reference plants were harvested at the same dates as the soybean plants.

Sampling preparation and analyses

Shoots and roots of soybeans were separated. Roots were washed to remove adhering soil. At the first sampling, nodules were counted and removed for determination of their dry weight. Soybean shoots and roots and the reference plants were dried at 45°C during 48 h. At harvest, the shoots were separated into stem, leaves, pods, and grains. Senescent leaves collected before the final harvest were pooled with the ones still hanging on the plants at maturity. The material of both soybean plants sampled per microplot was pooled. The dry weight of all plant parts was determined prior to crushing plant materials with a centrifuge mill (Granomat, Fuchs Maschinen AG, Switzerland) and milling them to powder (Pulverisette 5, Frisch, Germany). Total N content and atom% ^{15}N were analyzed on a continuous flow Roboprep CN Biological Sample Converter coupled to a tracermass mass spectrometer (Europa Scientific, England). The precision of this instrument was ±0.0002 atom%.

Calculations

The dilution of the ^{15}N taken up from the residual enriched fertilizer by ^{14}N derived from the atmosphere relative to that of non-fixing reference plants (weeds) was used to calculate the percentage of legume %Ndfa (in % of total plant N uptake) (McAuliffe et al. 1958):

Table 3 ^{15}N enrichment treatments (*EnrichTr*) applied in 2003 to the microplots that were in 2004 used for the ^{15}N enriched isotope dilution method

EnrichTr	^{15}N labeled component	Applied amount (g N/m^2)	Excess (atom% ^{15}N)
Urine	Sheep urine	12.7	8.1
Feces	Sheep feces	4.8	10.9
Mineral	NH_4NO_3	5.0	9.5
0N[a]	None	0	0

[a] Atom% ^{15}N abundance of soybean and reference plants grown in these microplots was used to calculate the atom% ^{15}N excess of soybean and reference plants, respectively, grown in the ^{15}N labeled microplots

$$\%\text{Ndfa} = \left[1 - \frac{\text{atom\% } ^{15}\text{N excess soybean}}{\text{atom\% } ^{15}\text{N excess reference plant}}\right] \times 100 \quad (1)$$

Calculations were carried out using the excess of the pooled soybean material and the average excess obtained for the weeds collected within one microplot.

The quantity of N_2 fixed (Nfix, g N/m^{-2}) is:

$$\text{Nfix} = \%\text{Ndfa} \times \text{TN}, \quad (2)$$

with: TN = total N taken up by soybean.

The atom% ^{15}N excess has been obtained by subtracting the NA of a relevant reference sample from the measured atom% ^{15}N of the enriched sample. As reference samples, we used plant materials that grew in microplots to which no ^{15}N enriched fertilizers had been applied (Bosshard 2006). If the atom% ^{15}N excess was obtained for n different soybean plant parts, a weighed average atom% ^{15}N excess (WAE) was calculated (Danso et al. 1993):

$$\text{WAE} = \frac{\sum_{i=1}^{n} \text{atom\% } ^{15}\text{N excess}_i \times \text{TN}_i}{\sum_{i=1}^{n} \text{TN}_i}, \quad (3)$$

where atom% ^{15}N excess$_i$ denotes the excess of a plant part and TN$_i$ its total N.

The WAE (Table 5) was used to calculate the %Ndfa at the whole plant level using Eq. 1. To enable comparison with published data and because roots could not be sampled completely, we present the %Ndfa and Nfix only for the shoot as obtained from all aboveground plant materials. The roots are considered only when mentioned

Table 4 Species and $\delta^{15}N$ (average and standard error) of reference plants sampled for the natural abundance technique in the plots of the different cropping systems

Species	Cropping system										Total	
	DYN		ORG		CON		MIN		NON			
	n	δ ^{15}N	*n*	δ ^{15}N	*n*	δ ^{15}N	*n*	δ ^{15}N	*n*	δ ^{15}N	*n*	δ ^{15}N
Agropyron repens			1	4.5	1	6.9	1	3.1			3	4.8 ± 1.1
Amaranthus lividus			1	11.4	1	4.7					2	8.1 ± 3.3
Apera spica-venti	1	5							1	3.6	2	4.3 ± 0.7
Chenopodium polyspermum					2	3.9 ± 0.5	2	4.1 ± 1.0			4	4.0 ± 0.5
Echinochloa crus-galli			1	3.2	2	3.1 ± 0.6	2	2.9 ± 0.3			5	3.0 ± 0.2
Glechoma hederaceum					2	2.7 ± 0.4	1	2.4			3	2.6 ± 0.2
Lolium perenne			1	3.8							1	3.8
Mentha arvensis									2	3.9 ± 0.7	2	3.8 ± 0.7
Plantago lanceolata									1	5.3	1	5.3
Poa annua									1	4.9	1	4.9
Polygonum aviculare	3	4.3 ± 0.2	3	5.3 ± 0.3	1	4.1	1	3.6	2	5.6 ± 1.2	10	4.8 ± 0.3
Polygonum persicaria	1	5.4	2	7.0 ± 1.4					7	4.7 ± 0.6	10	5.2 ± 0.6
Sonchus arvensis	5	4.1 ± 0.2	1	4.3			1	2.6			7	3.9 ± 0.3
Sonchus asper	5	5.0 ± 0.5	3	4.6 ± 0.1	1	2.6	4	3.0 ± 0.2	2	2.1 ± 0.4	15	3.9 ± 0.4
Stellaria media					2	4.5 ± 0.4	2	6.6 ± 1.9			4	5.2 ± 0.8
Taraxacum off			2	8.4 ± 0.0	3	4.2 ± 0.8	2	3.5 ± 0.2			7	5.2 ± 0.9
Total	15	4.6	15	5.9	15	4	16	3.6	16	4.4	77	4.5

Table 5 Atom% ^{15}N excess (average and standard error) of soybean and reference plants grown in the ^{15}N labeled microplots (*EnrichTr*) at late flowering and at maturity of soybean for an organic (*ORG*) and a conventional (*MIN*) cropping system (*CropSys*)

CropSys	Reference plant		Soybean		Difference	
	Late flowering (atom% ^{15}N)	Maturity (atom% ^{15}N)	Late flowering (atom% ^{15}N)	Maturity[a] (atom% ^{15}N)	Late flowering (atom% ^{15}N)	Maturity (atom% ^{15}N)
ORG						
Urine	0.16 ± 0.03	0.13 ± 0.01	0.12 ± 0.02	0.07 ± 0.02	0.057 ± 0.02	0.058 ± 0.03
Feces	0.14 ± 0.03	0.10 ± 0.01	0.06 ± 0.02	0.04 ± 0.01	0.079 ± 0.02	0.044 ± 0.02
Mineral	0.09 ± 0.02	0.06 ± 0.02	0.09 ± 0.02	0.04 ± 0.01	0.031 ± 0.02	0.028 ± 0.01
MIN						
Urine	0.17 ± 0.05	0.15 ± 0.07	0.18 ± 0.03	0.09 ± 0.02	0.013 ± 0.01	0.065 ± 0.02
Feces	0.12 ± 0.02	0.10 ± 0.02	0.11 ± 0.03	0.05 ± 0.00	0.022 ± 0.01	0.038 ± 0.02
Mineral	0.07 ± 0.02	0.05 ± 0.01	0.07 ± 0.02	0.05 ± 0.02	0.018 ± 0.01	0.011 ± 0.00
Anova source of variation						
CropSys	ns	ns	ns	ns	*	ns
EnrichTr	**	**	**	*	ns	ns
CropSys × EnrichTr	ns	ns	ns	ns	ns	ns

*, ** significant at 0.05, 0.01 probability level, respectively; ns = Not significant

[a] Presented values are weighed means according to Eq. 3

explicitly, e.g., in the discussion of the total fixed N amounts.

Natural abundance method

For the NA method, we sampled soybean and reference plants (weeds, Table 4) in plots of DYN, ORG, CON, MIN, and NON of the field experiment. We at random placed a frame with equal size as the microplots into the plots and harvested the plants of this area. Dates of sampling and the handling of the samples were exactly the same as for the ID method except that total N content and atom% ^{15}N were measured on a continuous flow ANCA-NT gas/solid/liquid preparation module coupled to a tracermass mass spectrometer (Europa Scientific; precision ± 0.2δ per mil). In case that the frame area contained less than two weeds, we collected them in the surrounding area.

Abundances are expressed in a relative, δ (delta) notation, which is the ‰ deviation of the 15N abundance of the sample from atmospheric N2 (=0.36637 atom% ^{15}N) (Unkovich et al. 1994):

$$\delta^{15}\mathrm{N}\,(‰) = \frac{\text{atom\% }^{15}\text{N sample} - \text{atom\% }^{15}\text{N air}}{\text{atom\% }^{15}\text{N air}} \times 1000. \quad (4)$$

The proportion of plant %Ndfa is (Shearer and Kohl 1986):

$$\%\mathrm{Ndfa} = \frac{\delta^{15}\mathrm{N}\ \text{reference plant} - \delta^{15}\mathrm{N}\ \text{soybean}}{\delta^{15}\mathrm{N}\ \text{reference plant} - B} \times 100. \quad (5)$$

The factor B refers to $\delta^{15}N$ value of the nodulated legume grown in media totally lacking N.

At maturity, a weighed $\delta^{15}N$ was calculated for soybean (Reiter et al. 2002):

$$\mathrm{W}\delta^{15}\mathrm{N} = \frac{\sum_{i=1}^{n} \delta^{15}\mathrm{N}_i \times \mathrm{TN}_i}{\sum_{i=1}^{n} \mathrm{TN}_i} \quad (6)$$

The W$\delta^{15}N$ (Table 6) was then used to calculate %Ndfa using Eq. 5.

For soybeans and weeds, respectively, calculations were again carried out with the average obtained for the soybean material collected within one frame and the two reference plants, respectively. Because B-values are affected by rhizobial strain and plant age and because they vary among different plant parts (Unkovich et al. 1994), we

Table 6 $\delta^{15}N$ (‰) (average and standard error) of soybean and reference plants grown in plots of the various cropping systems (*CropSys*) at late flowering and maturity of soybean

CropSys[a]	Reference plant		Soybean		Difference	
	Late flowering $\delta^{15}N$ (‰)	Maturity $\delta^{15}N$ (‰)	Late flowering $\delta^{15}N$ (‰)	Maturity[b] $\delta^{15}N$ (‰)	Late flowering $\delta^{15}N$ (‰)	Maturity $\delta^{15}N$ (‰)
DYN	4.9 ± 0.5	4.3 ± 0.4	3.0 ± 0.5	2.1 ± 0.5	1.2 ± 0.6	2.2 ± 0.3
ORG	5.3 ± 0.7	6.5 ± 1.5	2.1 ± 0.1	2.4 ± 0.5	3.0 ± 0.4	4.4 ± 1.2
CON	3.2 ± 0.3	4.6 ± 0.7	2.6 ± 0.1	2.1 ± 0.2	0.4 ± 0.3	2.4 ± 0.2
MIN	3.2 ± 0.4	4.0 ± 1.0	2.4 ± 0.5	2.7 ± 0.3	0.6 ± 0.4	1.3 ± 0.8
NON	4.7 ± 1.0	4.1 ± 0.9	2.5 ± 0.5	2.2 ± 0.2	1.8 ± 0.8	1.9 ± 0.3
Anova						
Source of variation						
CropSys	**	*	ns	ns	*	ns

*, ** significant at 0.05, 0.01 probability level, respectively; ns = Not significant

[a] For cropping systems see Table 1

[b] Presented values are weighed means according to Eq. 6

determined *B*-values in a pot experiment. Twenty pots filled with sand were sown with two inoculated soybean seeds per pot. The variety and inoculum were the same as used for the field grown soybean. To account for possibly different rhizobial genotypes in ORG and MIN soils, we additionally inoculated ten pots each with inoculum obtained from ORG or MIN soils, respectively. This inoculum was obtained by shaking 105 g field fresh soil from MIN and ORG plots, respectively, with 1.5 L sterile deionized water and sieving through a 20 µm steel net. Per pot 50 mL of this inoculate solution was added. Pots were daily watered with N free nutrient solution exactly composed as described in Unkovich et al. (1994). Five pots per treatment were harvested at the growth stage of late flowering/early pod filling, and five pots at the maturity of the plants. The separation into plant parts, sample preparation and analyses were done exactly as described for field grown soybean.

N balance

In 2004, no fresh N fertilizers were applied to soybean in any cropping system. Thus the N input–output balance accounting for deliberate N inputs into the cropping systems is the N input through symbiotic N_2 fixation minus the N withdrawal by soybean grains.

To calculate the N balance, Nfix of the whole plant, i.e., shoots and sampled roots, was used.

Statistical analysis

Statistical analyses were carried out using the GLM procedure of the statistical analysis package SYSTAT 10 (Systat Software Inc., Richmond, CA, USA). For analysis of variance percentage data was transformed using arcsin-transformation. For results of the ID technique, the effects of cropping system (CropSys), EnrichTr and the interaction of these two factors were tested. For the NA technique the influence of the cropping systems was tested. Pairwise comparisons were carried out by Fisher's least significant difference or *t*-test at $p = 0.05$.

Results and discussion

Isotopic composition of soil and plant N

The atom% ^{15}N excess of weeds and soybean was not different for plants grown on ORG or MIN but was influenced by the residual ^{15}N labeled fertilizers (Table 5). Due to its highest ^{15}N application (Table 3), excess was highest for the labeled urine treatment. For all three EnrichTrs, excess in plants and soils (Fig. 1) was at least 10% above NA and could be measured precisely with the mass spectrometer.

The excess in the plant materials is higher than the excess of soil N (Fig. 1). This shows that the residual fertilizer, which constitutes only a small

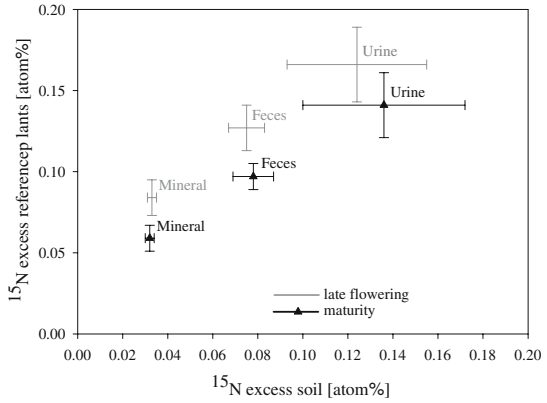

Fig. 1 ^{15}N excess of reference plants versus excess ^{15}N excess of total soil N for three different enrichment treatments (Urine, Feces, and Mineral). Reference plants were sampled at late flowering and maturity of soybean plants, respectively, and soil at maturity of soybean. *Error bars* indicate standard errors of the mean

proportion of the analyzed total soil N, still has a higher availability for the plants than native soil N. The excess measured in the reference plants reflects the order of the excess measured in the soil samples. Atom% ^{15}N excess in reference plants was on average 125% of atom% ^{15}N excess in soybean at late flowering, and 175% at maturity. However, variation in excess was high for both, reference plants and soybean. In some cases, particularly at late flowering, differences in excess atom% ^{15}N between reference plant and soybean were low or even zero.

The order of the $\delta^{15}N$ measured in the reference plants approximately reflects the order of $\delta^{15}N$ measured in the soil (Fig. 2). The $\delta^{15}N$ of total soil N ranged between 6.3 and 7.6 (means of differently cropped soils). Soils that were for 27 years under different cropping systems differentiate in their ^{15}N NA, which underlines the importance to sample reference plants in each cropping system. The two organic treatments ORG and DYN that had received exclusively animal manure (Table 1) have a higher $\delta^{15}N$ than the MIN soil that had received mineral fertilizers only, confirming findings of Gerzabek et al. (2001). It is known that animal excreta are enriched in ^{15}N relative to N_2 of the atmosphere (Heaton 1986) while mineral fertilizer ammonium is depleted and nitrate enriched (Freyer and Aly 1974). Also soil NON that had received no fertilizer inputs for the 27 years and where substantial amounts of soil N have been mineralized (Table 1) has a higher $\delta^{15}N$ than MIN. This can be explained by the fractionation during mineralization and because the mineralized N, with lower $\delta^{15}N$ than the substrate, is susceptible to plant uptake and loss from the soil (Hopkins et al. 1998; Amundson et al. 2003). This also explains why $\delta^{15}N$ values of reference plants, i.e., the $\delta^{15}N$ of available soil N, were for all cropping systems lower than of total soil N (means for differently cropped soils between 3.2 and 6.5‰, Table 6; Fig. 2). The $\delta^{15}N$ values of the reference plants were above the threshold of 2‰ required to apply the NA method when using an analytical precision in measurement of $\delta^{15}N$ of ± 0.2‰ (Unkovich et al. 1994). However, as seen for the ID method, variation was high (Table 6). Likewise, differences in $\delta^{15}N$ between reference plants and legumes were in some cases low. For both methods, low differences between reference plants and soybean indicate low-symbiotic N_2 fixation rates. At low-fixation rates, errors of both methods increase (Danso et al. 1993; Högberg 1997).

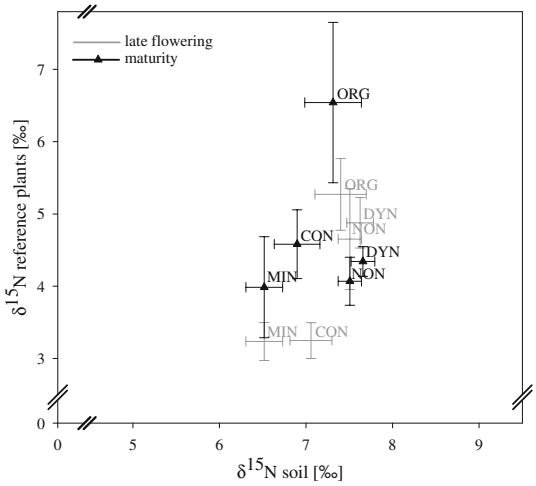

Fig. 2 $\delta^{15}N$ of reference plants versus $\delta^{15}N$ of soil for organic (DYN, ORG) and conventional (CON, MIN) cropping systems and the non-fertilized control soil (NON). Reference plants were sampled at late flowering and maturity of soybean plants, respectively, and soil at maturity of soybean. *Error bars* indicate standard errors of the mean

For both, the ID and the NA method, variation in excess atom% ^{15}N and $\delta^{15}N$, respectively, in the reference plants and in soybean was high (Tables 5, 6). This suggests a high-spatial variability in the isotopic composition of available soil N. For this reason, the inclusion of a variety of reference plants is being recommended, particularly for the NA method (Högberg 1997). We found no clear pattern in $\delta^{15}N$ for dicotyledonous of monocotyledonous weeds (Table 4). Also within a variety $\delta^{15}N$ varied broadly. Still, a standardized procedure including the same group of weeds in each treatment and/or more samples taken from larger areas might have lowered the variation of our results. However, we were limited by space in the microplots and by minimal intervention principles in the main plots of the field experiment.

B-values

The *B*-value for soybean shoots harvested at late flowering was –1.172‰ (± 0.118). It was not significantly different for plants grown with ORG or MIN inoculum. In contrast, at maturity, *B*-values differed not only between plant organs but the average *B*-value for the shoot for ORG (–0.876‰ ± 0.069) was also significantly ($p < 0.01$) different from the *B*-value for MIN (–0.492‰ ± 0.060) (Fig. 3). Thus, the corresponding *B*-values were used for the calculation of the %Ndfa in the shoots using Eqs. 5 and 6.

The isotopic fractionation of ^{15}N in different plant organs is well known (Werner and Schmidt 2002; Okito et al. 2004). As the *B*-values were obtained under otherwise identical experimental conditions, the differences found for ORG and MIN at maturity suggest that the inoculum obtained from these two soils might have contained different genotypes of *R. japonicum*. The effect of rhizobial strain on the *B*-value has been repeatedly reported, e.g., by Unkovich et al. (1994) for *Trifolium subterraneum* cv. Trikkala and by Oktio et al. (2004) for soybean.

Our *B*-values are in the range reported for several herbaceous and woody legumes (Unkovich et al. 1994; Gathumbi et al. 2002). Bergersen et al. (1985) report a *B*-value of 0.546‰ for entire soybean plants, but *B*-values are lower for shoots because they are more ^{15}N depleted (Fig. 3; Okito et al. 2004).

Dry matter production and total N uptake by soybean

At maturity, grain production ranged from 561 to 917 g/m^2, and total dry matter production from 1,260 to 2,190 g/m^2 area (Table 7). There was no significant effect of the cropping systems, but yields were lowest for NON. Grains ever constituted more than 40% to the total dry matter.

Dry matter production was not affected by the ^{15}N labeled EnrichTr applied to the microplots of the ID method. Therefore, mean values of ORG and MIN, respectively, are presented (Table 7). However, dry matter production was lower in the microplots of the ID method than in equal areas sampled for the NA method. This suggests that the growth of the soybean plants of the ID method was restricted by the microplot frames. Indeed, over the complete vegetation period soybeans of the ID method growing in the microplots were smaller than the ones growing in ORG and MIN plots, respectively. Still, grain production of plants sampled for the NA and the ID method was higher than grain yield obtained for the complete, threshing machine harvested, ORG and MIN plots of the

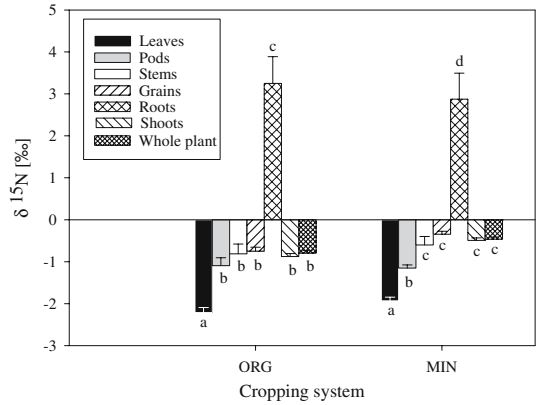

Fig. 3 $\delta^{15}N$ (*B*-value) of different plant parts at maturity of soybean grown on N free media with inoculate from soil that was for 27 years under bio-organic (ORG) or conventional (MIN) cropping systems, respectively. Values are uncorrected for $\delta^{15}N$ [‰] of the sown seeds, which was –0.85. *Error bars* indicate standard errors of the mean

Table 7 Dry matter production of soybean at maturity grown under different cropping systems (*CropSys*), used for the isotope dilution (*ID*) or the natural abundance (*NA*) technique

Technique/CropSys[a]	Plant part					
	Leaves (g/m^2)	Pods (g/m^2)	Grains (g/m^2)	Stem (g/m^2)	Roots[b] (g/m^2)	Sum (g/m^2)
ID						
ORG	113	269	564	255	83	1283
MIN	106	300	561	271	94	1332
SEM	12	52	105	38	16	196
Anova						
Source of variation						
CropSys	ns	ns	ns	ns	ns	ns
NA						
DYN	56 a	391	834	518 bc	128	1930
ORG	140 b	311	670	413 ab	91	1630
CON	78 ab	411	773	521 bc	116	1900
MIN	75 a	450	917	642 c	110	2190
NON	47 a	273	561	302 a	76	1260
SEM	21	49	100	53	12	204
Anova						
Source of variation						
CropSys	*	ns	ns	**	ns	ns

*, ** significant at 0.05, 0.01 probability level, respectively; ns = Not significant

[a] For cropping systems see Table 1

[b] Only partial root sampling

field trial. This can be explained by no grain losses with the manual harvest applied for the ID and NA technique. Therefore, the results presented in Table 7 as well as all data relating to frame areas (expressed in m^2) cannot be extrapolated to larger scales.

Effects of methods and cropping systems on the N accumulation in soybean agree with the ones observed for dry matter production (Fig. 4). Total N uptake was lowest in NON (39 g/m^2) and highest in MIN (69 g/m^2). Grains contained 87–90% of total N, except for MIN sampled for the NA technique where the grain N proportion was

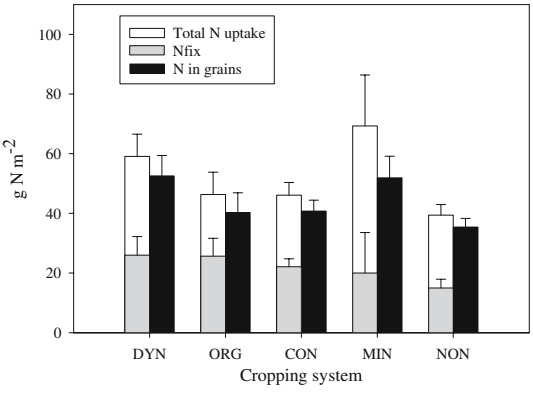

Fig. 4 Quantities of symbiotically fixed N_2 (*Nfix*) in shoot and root, total N uptake and N contained in grains of mature soybean grown in organic (DYN, ORG) and conventional (CON, MIN) cropping systems or on a control that was not fertilized for 27 years (NON). *Error bars* indicate standard errors of the mean

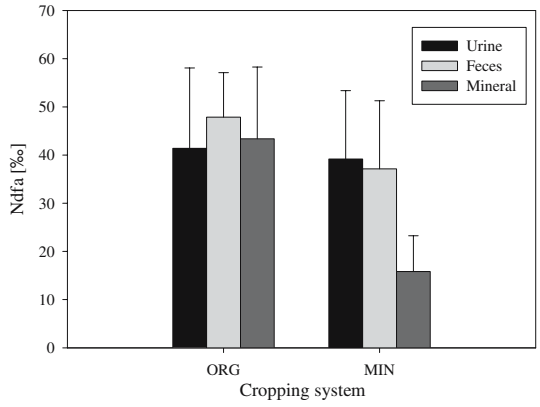

Fig. 5 %Ndfa of soybean at maturity obtained with different residual enrichment treatments (^{15}N labeled urine, feces or mineral N, respectively). *Error bars* indicate standard errors of the mean

only 75%. The average N content for all plants was 31 g/kg dry matter for the shoot and 60 g/kg dry matter for the grains.

Comparison of enrichment treatments

Over the complete dataset the %Ndfa values obtained by the three different EnrichTrs (urine, feces or mineral N) were not significantly different (Fig. 5). This shows that 1 year after application, enrichment by ^{15}N labeled N feces was not superior to enrichment by the less expensive ^{15}N labeled mineral N fertilizer. The use of residual ^{15}N labeled mineral N for studying symbiotic N_2 fixation is known (McNeill et al. 1998; Vinther and Jensen 2000). Still, freshly applied ^{15}N labeled feces N could present a useful EnrichTr because feces is a slow release N source (Sorensen et al. 1994).

Because total N accumulation did not differ between the three EnrichTrs, also Nfix was equal (data not shown). Therefore, to compare the %Ndfa obtained by the ID and NA methods, the averages for ORG and MIN, respectively, of the three EnrichTrs were used.

Comparison of ID and NA method

The %Ndfa obtained with the ID method was for both sampling dates not significantly different from the %Ndfa obtained with the NA method (Fig. 6), confirming other studies where these two methods were found to be on a par (Carranca et al. 1999; Huss-Danell and Chaia 2005). The %Ndfa obtained by ID and NA also showed similar high variation. Because (1) the %Ndfa of the ID and NA techniques are comparable, (2) growth of soybeans of the ID method was restricted by the microplot frames, and (3) more cropping systems could be investigated with the NA than the ID technique, we henceforth only discuss the results obtained with the NA method.

Percentage of legume N derived from the atmosphere

At late flowering, which usually is the more suitable sampling date than maturity because N uptake has not yet ceased (Bergersen et al. 1985; Drevon 1997; Unkovich and Pate 2000) mean %Ndfa values lay between 8 and 45%. The lowest %Ndfa for both conventional systems CON and MIN were significantly different from the highest %Ndfa of ORG. With 20 and 27%, respectively, DYN and NON took intermediate positions. At plant maturity, the %Ndfa was for all cropping systems significantly higher than at the first sampling (Fig. 6). The lowest %Ndfa of the CON system MIN (24%) was significantly lower than the highest %Ndfa of ORG (54%). In

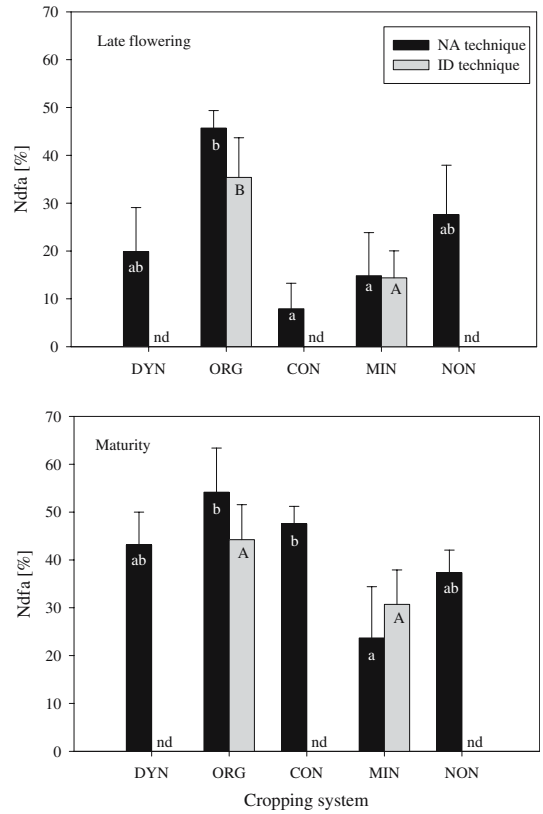

Fig. 6 Percentage of legume N derived from the atmosphere (%Ndfa) of soybean shoot grown in organic (DYN, ORG) and conventional (CON, MIN) cropping systems and on a control soil that was not fertilized for 27 years (NON) at late flowering and maturity of soybean. %Ndfa was estimated by natural abundance (NA) or enriched ^{15}N isotope dilution (ID) technique. Error bars indicate standard errors of the mean. Different lower and upper case letters indicate significant differences of %Ndfa between cropping systems investigated by the NA and ID technique, respectively (LSD test, $p < 0.05$)

Table 8 Macronutrient content in soybean shoots grown under different cropping systems (*CropSys*) at late flowering/early pod-filling

CropSys[a]	N[b] (g/kg)	P[c] (g/kg)	K[c] (g/kg)	Ca[c] (g/kg)	Mg[c] (g/kg)
DYN	20.1	2.4 a	10.1 ab	14.7	4.5
ORG	21.9	2.5 ab	12.5 a	15.2	4.3
CON	18.2	2.6 ab	14.7 c	14.4	4.2
MIN	20.0	2.9 b	15.4 c	16.5	4.5
NON	21.0	2.2 ab	8.8 bc	16.5	5.3
SEM	2.2	0.3	0.2	1.2	0.8
Anova					
Source of variation					
CropSys	ns	*	**	ns	ns

*, **, *** significant at 0.05, 0.01, and 0.001 probability level, respectively; ns = Not significant; means followed by the same letter are not significantly different (Fisher's LSD, $p < 0.05$)

[a] For cropping systems see Table 1

[b] Europa Scientific Roboprep CN coupled to a Tracermass mass spectrometer (Europa Scientific, England)

[c] Determined by calcinations and subsequent HNO_3-extraction (Gallet et al. 2003)

contrast to the first sampling date, the conventional system CON also had a high %Ndfa.

At both sampling dates, the %Ndfa was rather low. %Ndfa values of soybeans can vary from 0 to 95% (Unkovich and Pate 2000), but for soybean cropped without fresh N fertilization, like in our study, %Ndfa greater than 70% has been reported (Alves et al. 2003). With an overall average of 27 well-developed nodules per plant (0.16 g dry matter per nodule) at late flowering/early pod filling, plants were according to Drevon (1997) well nodulated. There were no significant differences between cropping systems for nodule number and nodule dry matter. However, the soil of the field trial has a high soil N mineralization potential which is illustrated, e.g., by a cumulated N withdrawal of more than 3,600 kg N/ha over 26 years of cultivation without any fertilizer application in NON (Table 1). Thus high-soil mineral N supply may have limited symbiotic N_2 fixation (Herridge and Brockwell 1988). Furthermore, the interpretation scheme of the analyzed soil nutrient contents (Walther et al. 2001) shows that available P is very low in NON and low in DYN and ORG soils, and K contents are low to very low in all studied soils (Table 2). Low P and K contents in the soil translated into P and K contents in the soybean plants grown on NON and DYN soils (Table 8) that were below values considered as adequate (Reuter and Robinson 1986). Low-P availability is known to reduce %Ndfa (see literature compilation in Hartwig 1998).

Quantity of N_2 fixed and N balance of soybean

The total amount of N fixed at maturity was between 15 and 26 g/m^2, with the minimum for NON and the maximum for DYN (Fig. 4). Because of the high variation, there were no significant differences between the cropping systems which took the order DYN > ORG > CON > MIN > NON. Differences in total N uptake were greater than for the quantity of Nfix. As no fresh fertilizer was applied to any cropping system, soil N accounted for this difference, which was non-significantly greater in MIN and DYN than the other systems. The results obtained for ORG and MIN with the ID method show that the residual contribution of fertilizers applied in 2003 to winter wheat to total soybean N uptake was less than 1.5% (Bosshard 2006).

The N withdrawal by harvested grains was for all cropping systems greater than the N input through symbiotic N_2 fixation (Fig. 4), resulting in a negative N balance of –14 to –30 g/m^2. This N balance does not account for all root N because the root system was only partly recovered and because N exuded into the soil or derived from dying roots is not included. However, in our case N withdrawal by grains would still exceed Nfix if we would assume that the non-recoverable root N

accounts for as much as 30–35% of total plant N (Alves et al. 2003).

This means that irrespective of the cropping system, soybean resulted in a net removal of N from the soil in spite of symbiotic N_2 fixation. This net N removal was greater the higher the grain yield, because the amount of symbiotically fixed N varied less between the cropping systems than N uptake in grains. Therefore, N withdrawal from the soil was greatest for MIN and lowest for ORG.

Conclusions

The %Ndfa obtained with the ID method was not significantly different from the %Ndfa obtained with the NA method, confirming other studies were these two methods were found to be on a par. As the growth of soybeans of the ID method was restricted by the microplot frames, NA gave more accurate results for Nfix than the ID method. Furthermore, when used in a long-term field experiment like the one used for our study, the NA method permits more extended work than the ID method, e.g., in terms of systems or areas included as nothing needs to be added or to be disturbed.

Regarding the EnrichTrs of the ID method, we found no advantage of using ^{15}N labeled animal manure compared to mineral N in the second year after application. This does not preclude that ^{15}N labeled animal manure would be a useful option if applied freshly, e.g., to study symbiotic N_2 fixation by legumes in organically fertilized systems.

At the low to medium Ndfa found for all treatments, soybeans did not improve the N supply to the system but removed significant amounts of soil N. The high-N mineralization potential of the soil at the field site may explain this finding. In addition, in spite that plants were well nodulated, the efficiency of the symbiosis in the DYN, ORG, and NON soils might have been limited by low-soil P availability.

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References

Alves BJR, Boddey RM, Urquiaga S (2003) The success of BNF in soybean in Brazil. Plant Soil 252:1–9

Amundson R, Austin AT, Schuur EAG, Yoo K, Matzek V, Kendall C, Uebersax A, Brenner D, Baisden WT (2003) Global patterns of the isotopic composition of soil and plant nitrogen. Glob Biogeochem Cycle 17:1031

Bergersen FJ, Turner GL, Gault RR, Chase DL, Brockwell J (1985) The natural abundance of N-15 in an irrigated soybean crop and its use for the calculation of nitrogen fixation. Aust J Agric Res 36:411–423

Besson JM, Niggli U (1991) DOK-Versuch: vergleichende Langzeit-Untersuchungen in den drei Anbausystemen biologisch-dynamisch, organisch-biologisch und konventionell:1. Konzeption des DOK-Versuches: 1. und 2. Fruchtfolgeperiode Schweiz Landw Forsch 31:79–109

Bosshard C (2007 in preparation) Nitrogen dynamics in organic and conventional cropping systems. Swiss Federal Institute of Technology Zurich, ETH Zurich

Carranca C, de Varennes A, Rolston DE (1999) Biological nitrogen fixation estimated by N-15 dilution, natural N-15 abundance, and N difference techniques in a subterranean clover-grass sward under Mediterranean conditions. Eur J Agron 10:81–89

Danso SKA, Hardarson G, Zapata F (1993) Misconceptions and practical problems in the use of 15N soil enrichment techniques for estimating N2 fixation. Plant Soil 152:25–52

Davidson EA, Hart SC, Shanks S, Firestone MK (1991) Measuring gross nitrogen mineralization, immobilization, and nitrification by ^{15}N isotopic pool dilution in intact soil cores. J Soil Sci 42:335–349

Dirks B, Scheffer H (1930) Der Kohlensäure-bikarbonatauszug und der Wasserauszug als Grundlage zur Ermittlung der Phophorsäurebedürftigkeit der Böden. Landwirtschaftliche Jahrbücher 71:73–99

Drevon JJ (1997) Nodular diagnosis. In: Morot-Gaudry J-F (ed) Nitrogen assimilation by plants. INRA, UK, pp 417–426

Freyer HD, Aly AIM (1974) Nitrogen-15 variations in fertilizer nitrogen. J Environ Qual 3:405

Gallet A, Flisch R, Ryser JP, Nosberger J, Frossard E, Sinaj S (2003) Uptake of residual phosphate and freshly applied diammonium phosphate by Lolium perenne and Trifolium repens. J Plant Nutr Soil Sci-Z Pflanzenernahr Bodenkd 166:557–567

Gathumbi SM, Cadisch G, Giller KE (2002) N-15 natural abundance as a tool for assessing N-2-fixation of

herbaceous, shrub and tree legumes in improved fallows. Soil Biol Biochem 34:1059–1071
Gerzabek MH, Haberhauer G, Kirchmann H (2001) Nitrogen distribution and N-15 natural abundances in particle size fractions of a long-term agricultural field experiment. J Plant Nutr Soil Sci-Z Pflanzenernahr Bodenkd 164:475–481
Giller KE (2001) Nitrogen fixation in tropical cropping systems. CABI, Wallingford, Oxon, UK
Hansen B, Kristensen ES, Grant R, Hogh-Jensen H, Simmelsgaard SE, Olesen JE (2000) Nitrogen leaching from conventional versus organic farming systems—a systems modelling approach. Eur J Agron 13:65–82
Hartwig UA (1998) The regulation of symbiotic N2 fixation: a conceptual model of N feedback from the ecosystem to the gene expression level. Perspecitves Plant Ecol Evol Syst 1:92–120
Heaton THE (1986) Isotopic studies of nitrogen pollution in the hydrosphere and atmosphere—a review. Chem Geol 59:87–102
Herridge DF, Brockwell J (1988) Contributions of fixed nitrogen and soil nitrate to the nitrogen economy of irrigated soybean. Soil Biol Biochem 20:711–717
Högberg P (1997) ^{15}N natural abundance in soil-plant systems. New Phytol 137:179–203
Hopkins DW, Wheatley RE, Robinson D (1998) Stable isotope studies of soil nitrogen. In: Griffiths H (ed) Stable isotopes: integration of biological, ecological and geochemical processes. BIOS Scientific Publisher Ltd., Oxford, pp 75–88
Huss-Danell K, Chaia E (2005) Use of different plant parts to study N-2 fixation with N-15 techniques in field-grown red clover (Trifolium pratense). Physiol Plant 125:21–30
KIP (1999) Richtlinien für den ökologischen Leistungsnachweis (ÖLN). Koordinationsgruppe Richtlinien Deutschschweiz, Landwirtschaftliche Beratungszentrale Lindau
Kirchmann H, Bergstrom L (2001) Do organic farming practices reduce nitrate leaching? Commun Soil Sci Plant Anal 32:997–1028
Langmeier M, Frossard E, Kreuzer M, Mäder P, Dubois D, Oberson A (2002) Nitrogen fertilizer value of cattle manure applied on soils originating from organic and conventional farming systems. Agronomie 22:789–800
Mäder P, Fließbach A, Dubois D, Gunst L, Fried P, Niggli U (2002) Soil fertility and biodiversity in organic farming. Science 296:1694–1697
McAuliffe C, Chamblee DS, Uribe-Arango H, Woodhouse WW (1958) Influence of inorganic nitrogen on nitrogen fixation by legumes as revealed by 15N. Agron J 50:334–337
McNeill AM, Pilbeam CJ, Harris HC, Swift RS (1998) Use of residual fertiliser ^{15}N in soil for isotope dilution estimates of N_2 fixation by grain legumes. Aust J Agric Res 49:821–828
Okito A, Alves B, Urquiaga S, Boddey RM (2004) Isotopic fractionation during N-2 fixation by four tropical legumes. Soil Biol Biochem 36:1179–1190
Reiter K, Schmidtke K, Rauber R (2002) The influence of long-term tillage systems on symbiotic N2 fixation of pea (Pisum sativum L.) and red clover (Trifolium pratense L.). Plant Soil 238:41–55
Reuter DJ, Robinson JB (1986) Plant analysis: an interpretation manual. Inkata Press, Melbourne, 218pp
Schwenke GD, Peoples MB, Turner GL, Herridge DF (1998) Does nitrogen fixation of commercial, dryland chickpea and faba bean crops in north-west New South Wales maintain or enhance soil nitrogen? Aust J Exp Agric 38:61–70
Shearer G, Kohl DH (1986) N2 fixation in field settings: estimations based on natural abundance. Aust J Plant Physiol 13:699–744
Siegrist S, Schaub D, Pfiffner L, Mäder P (1998) Does organic agriculture reduce soil erodibility? The results of a long-term field study on loess in Switzerland. Agric Ecosyst Environ 69:253–264
Sorensen P, Jensen ES, Nielsen NE (1994) Labelling of animal manure nitrogen with 15N. Plant Soil 162:31–37
Stockdale EA, Lampkin NH, Hovi M, Keatinge R, Lennartsson EKM, Macdonald DW, Padel S, Tattersall FH, Wolfe MS, Watson CA (2001) Agronomic and environmental implications of organic farming systems. Adv Agron 70:261–327
Thomsen IK, Schjønning P, Christensen BT (2003) Mineralisation of 15N-labelled sheep manure in soils of different texture and water contents. Biol Fertil Soils 37:295–301
Unkovich MJ, Pate JS (2000) An appraisal of recent field measurements of symbiotic N-2 fixation by annual legumes. Field Crop Res 65:211–228
Unkovich MJ, Pate JS, Sanford P, Armstrong EL (1994) Potential precision of the d15N natural abundance method in the field estimates of nitrogen fixation by crop and pasture legumes in south-west Australia. Aust J Agric Res 45:119–132
Vance ED, Brookes PC, Jenkinson DS (1987) An extraction method for measuring soil microbial biomass C. Soil Biol Biochem 19:703–707
Vinther FP, Jensen ES (2000) Estimating legume N2 fixation in grass-clover mixtures of a grazed organic cropping system using two 15N methods. Agric Ecosyst Environ 78:139–147
Walther U, Ryser J-P, Flisch R (2001) Grundlagen für die Düngung im Acker- und Futterbau 2001. Agrarforschung 8:1–80
Werner RA, Schmidt HL (2002) The in vivo nitrogen isotope discrimination among organic plant compounds. Phytochemistry 61:465–484