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USE OF ^{15}N ENRICHED PLANT MATERIAL FOR LABELLING OF SOIL NITROGEN IN LEGUME DINITROGEN FIXATION EXPERIMENTS

E.S. Jensen

Abstract. The soil nitrogen in a field plot was labelled with nitrogen-15 (^{15}N) by incorporating labelled plant material derived from previous experiments. The nitrogen added in plant material corresponded to approx. 3% of total N present in the top soil (0-20 cm). The ^{15}N enrichment of the plant N was approx. 1 atom % ^{15}N. The plot was used the following 3 years for determination of the amount of N_2 fixed by different leguminous plants.

The atom % ^{15}N excess in grains of cereals grown as reference crops was 0.20, 0.05 and 0.03 in the 3 years, respectively. In the first year the level of enrichment was adequate for estimating symbiotic nitrogen fixation. In the second and third year lack of precision in determination of the $^{15}N/^{14}N$ ratios of legume N, may have caused an error in estimates of nitrogen fixation. In the first year 77% of the nitrogen taken up in a pea crop was derived from nitrogen fixation.

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Four years after incorporating the labelled plant material, the ^{15}N enrichment of the plant available soil N, released by mineralization, was 0.04. The enrichment of the inorganic N pool approached thus a stable level after 2 to 3 years. The enrichment of the total soil was, however, only 0.014 atom % ^{15}N excess.

About 23% of the labelled N was taken up by plants during the 3 years of cropping; after 4 years about 44% of the labelled N was found still to be present in the top soil.

The labelling of the soil nitrogen with organic bound ^{15}N, compared to adding mineral ^{15}N at sowing, is advantageous because the labelled N is released by mineralization so that the enrichment of the plant available soil N pool become more uniform during the growth season; and high levels of mineral N, which may depress the fixation process, is avoided.

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1. INTRODUCTION

The most reliable methods for quantifying symbiotic nitrogen fixation in the field involves the use of the stable nitrogen isotope ^{15}N. Direct measurement of N_2 fixation under field conditions requires the use of gas lysimeters and expensive $^{15}N_2$ (WAREMBOURG et al., 1982; SIMS et al., 1983). Nitrogen fixation can also be determined indirectly by labelling the plant available soil N pool with ^{15}N. When this method is used a non-fixing plant has to be grown simultaneously (FRIED and BROESHART, 1975).

In most studies involving the use of the indirect isotope dilution technique, the plant available soil N pool is labelled either by one addition of ^{15}N enriched or depleted N fertilizer at the time of planting (FRIED and BROESHART, 1975; WITTY, 1983; JENSEN, 1987; CLAIR et al., 1988), by adding slow-release fertilizer (WITTY, 1983; WITTY and RITZ, 1984), or by several small additions during growth (MCAULIFFE et al., 1958; VALLIS et al., 1967). The soil N may also be labelled by incorporation of ^{15}N in the more or less mineralizable fractions of soil organic matter; either by addition of the mineral ^{15}N together with carbon sources like sucrose and straw (LEGG and SLOGER, 1975; FALK, 1980; CHALK et al., 1983; GILLER and WITTY, 1987) or by adding labelled organic material (FRIED et al., 1983; DE FREITAS et al., 1984; WITTY and RITZ, 1984). If ^{15}N is incorporated in the soil organic matter it requires that the soil must be treated several months before planting, since it is nescessary to await mineralization of the organically bound ^{15}N.

Independent of the method used for labelling the plant available soil N, several assumptions underlies the estimation of nitrogen fixation from legume and reference crop data. These matters have been thoroughly dicussed by CHALK (1985), VOSE and VICTORIA (1986) and DANSO (1988). One of the important assumptions are that the ratio between indigenous and labelled soil N taken up should be the same in fixing and non-fixing plant (FRIED and BROESHART, 1975). Ideally the crops should be able to exploit identical soil volumes for mineral N and also have identical pat-

terns of mineral N uptake, especially if the enrichment of the soil N pool changes during the growth season (WITTY, 1983). These requirements make it difficult to select the appropriate reference crop (WITTY, 1983). A method proposed by LEDGARD et al. (1985), in which inorganic fertilizer N with different ^{15}N enrichments are included, may be used for selecting the most suitable reference crop.

However, a reference crop which does not perfectly match the legume crop in pattern of soil mineral N uptake, can be used if it is possible uniformly to label the soil N to the rooting depth of the deeper rooting species and label it in such a way that the enrichment of the plant available soil N is stable during the growth season. Incorporation of ^{15}N in the soil organic matter creates a slow release ^{15}N label, and thereby presumably a more stable enrichment of the plant available soil N pool as compared to the enrichment resulting from addition of mineral ^{15}N at sowing. Addition of mineral N has also the disadvantage, that added mineral N may significantly influence the N_2 fixation process (JENSEN, 1986a).

The aim of the present study was to evaluate soil incorporation of ^{15}N labelled plant material as a method for labelling the soil N in field experiments established for measuring symbiotic dinitrogen fixation in legumes.

2. MATERIAL AND METHODS

2.1. Plot preparation

A field plot (4.5 x 4.5 m) was prepared in November 1983 on a sandy loam soil (0.13% N, 1.1% C) at Risø. The plot was thoroughly rotary tilled to 20 cm soil depth before adding the organic material. The material was a mixture of finely milled pea seed, pea straw and spring barley straw. The mixture contained 1.88%

N; assuming a C concent of 40% the C/N was approx. 21. The material contained 1.318 atom % ^{15}N. Material was added to soil at a rate of 532 g m^{-2} corresponding to 10 g N m^{-2} (100 kg N ha^{-1}) using a grid of 1 m^2. The material was then incorporated in the upper 20 cm of the soil profile.

Three weeks later and in April 1984 soil was sampled from the amended layer for determination of KCl extractable N and ^{15}N enrichment. In the autumn 1987 soil was sampled for determination of total N and ^{15}N enrichment.

In 1984, 1985 and 1986 the plot was cropped with legumes and reference crops as described below. The plot received PK fertilizer corresponding to 3 g P and 5 g K m^{-2} each year. Weeds were removed by hand and plant pathogens were controlled with fungicides.

2.2. Experiment 1

The following plant species were sown early May 1984:

N_2-fixing crops:
- Field pea (Pisum sativum L.) cv. 'Frisson'
- Field pea (Pisum sativum L.) cv. 'Afghanistan', derived from Dr. T.A. Lie, Wageningen, Holland

Reference crops:
- Non-nodulating (nod^-) mutant of 'Frisson' pea derived from Dr. Messager, Dijon, France
- Spring oilseed rape (Brassica napus oleifera L.) cv. 'Line'
- Winter oilseed rape (Brassica napus oleifera L.) cv. 'Jupiter'
- Spring barley (Hordeum vulgare L.) cv. 'Nery'

Each crop was grown in five rows of lenght 4.5 m at a row distance of 15 cm. Above-ground plant parts were harvested 5, 8 and 14 (maturity) weeks after seedling emergence.

2.3. Experiment 2

Winter vetch (Vicia villosa L.) cv. 'Ostsaat Dr. Baumanns' and winter wheat (Triticum aestivum L.) cv. 'Kansler' were sown in September 1984 after thoroughly rotary tilling. Each plot consisted of 10 rows, lenght 4.5 m and 15 cm row distance. The crops were harvested at maturity by cutting plant from 2 x 1 m^2 at the soil surface.

2.4. Experiment 3

In April 1986 white lupin (Lupinus albus L.) cv. 'Lublanc' was sown in two plots of five rows each (30 cm row distance). Spring wheat (Triticum aestivum L.) cv. 'Walter' were sown in a plot consisting of 10 rows with 15 cm row distance. One of the lupin plots was inoculated with Rhizobium lupinii by spraying 600 ml yeast mannitol broth containing approx. 10^9 cells ml^{-1} suspended in 10 l of water on the plot. The crops were harvested late September. Lupin plants had not matured completely and a large loss of wheat seeds had occured at this late harvest.

2.5. Chemical analysis and calculations

The dry mater production of crops was determined after drying in an well-areated oven at 80°C for 20 h. Total N in the plant material was determined by a semi-micro Kjeldahl procedure, which included nitrate (BREMNER and MULVANEY, 1982). Data for dry matter and nitrogen presented in tables are means of two replicates. The ^{15}N enrichment of ammonium in the pooled Kjeldahl digests were determined by mass spectrometry as described in detail by JENSEN (1986b). The atom % ^{15}N excess was obtained by subtracting 0.3664 from the atom % ^{15}N.

The per cent of nitrogen in legumes derived from symbiotic nitrogen fixation (% Ndfa) was estimated as described by FRIED and MIDDELBOE (1977) using the atom % ^{15}N excess, which is corrected for seed-borne N according to JENSEN et al. (1985). If ^{15}N analy-

sis was carried out on individual plant parts, weighed atom % ^{15}N were calculated to obtain enrichments for the total plant N. The equation was:

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

The amount of N_2 fixed was obtained by multiplying the %Ndfa with total plant N. The per cent of N in plants which was derived from the labelled plant material (%Ndfpm) was calculated as:

$$\%Ndfpm = \left[\frac{\text{atom \% } ^{15}N \text{ excess, crop}}{\text{atom \% } ^{15}N \text{ excess, material}}\right] \times 100 \quad (2)$$

The per cent of total N in plants derived from the soil (%Ndfs) was calculated as:

$$\%Ndfs = 100 - \%Ndfa - \%Ndfpm - \%Ndfk \quad (3)$$

where %Ndfk is the per cent of plant N derived from the seeds.

The amount of nitrate and ammonium in the soil was determined in 2 M KCl extracts (soil:KCl, 1:10) using distillation and titration. Total N in soil was determined by elemental analysis using a Carlo Erba NA1500 Analyzer and 50 mg samples.

Decomposition of the added material was studied in a laboratory incubation experiment. Soil samples of 114 g (100 g dry soil) in 250 ml Erlenmeyer flasks were amended with 233 mg plant material (93.2 mg C og 4.38 mg N flask^{-1}) and incubated at 20°C for 94 days. The CO_2 production during incubation was determined according to SØRENSEN (1974) and net mineralization was determined after 94 days of incubation.

3. RESULTS

3.1. Experiment 1

Dry matter production and nitrogen content of crops from Exp. 1 are shown in Table 1. 'Afghanistan' pea was initialy included in the experiment as a reference crop, since it had been observed that this primitive pea cultivar did not normally nodulate with European strains of Rhizobium. In this experiment a few and large nodules were unexpectedly formed, which also fixed nitrogen (JENSEN et al., 1986). Consequently, 'Afghanistan' pea could not be used as a reference crop. Because of the late and sparse nodulation in 'Afghanistan' the dry matter production and nitrogen accumulation was much smaller than in 'Frisson' pea (Table 1).

Growth and nitrogen uptake in the four reference crops were markedly different with spring barley being the higher yielding crop (Table 1). The growth of the non-nodulating mutant was very poor indicating that not only nodulation have been affected by the mutation. A significant increase in the accumulated N from harvest at week 8 to week 14 at maturity was found only in the nitrogen fixing crops (Table 1). A loss of N occured in reference crops, probably due to loss of dead leaves. Since plants were not vernalized, no seeds were produced in the winter oilseed rape. The purpose of having winter oilseed rape in the experiment was to include a crop which did not enter the reproductive growth stage, and therefore continued to take up soil N.

The atom % ^{15}N excess in 'Frisson' pea was significantly lower than in the reference crops (Table 2). Since 'Afghanistan' nodules were formed rather late in the growth season a reduction in the atom % ^{15}N compared to references was not observed before the final harvest (Table 2). The atom % ^{15}N in reference crops decreased during growth and at the final harvest the enrichment was about 0.19 atom % ^{15}N excess (Table 2). Without correction for seed-borne N the difference in ^{15}N enrichment of references at the first harvest varied between 0.188 and 0.285 atom % ^{15}N excess. The range of ^{15}N enrichments was reduced after correction

for seed-borne N to from 0.219 to 0.286 atom % ^{15}N excess. At the later growth stages the seed N correction had no significant influence on the atom % ^{15}N excess (JENSEN et al., 1986).

Table 1. Above-ground biomass yield and nitrogen content of crops from Exp. 1.

Crop	Weeks after emergence			
	5	8	14	
			Straw	Seed
	Dry matter yield, g m^{-2}			
N_2 fixing crop				
Frisson pea	48	440	256	346
Afghanistan pea	26	184	146	94
Reference crop				
Nod$^-$ pea	31	87	68	20
Spring oilseed rape	57	237	249	18
Winter oilseed rape	166	331	301	-
Spring barley	178	588	411	376
	Nitrogen content, g N m^{-2}			
N_2 fixing crop				
Frisson pea	1.6	12.8	3.0	13.3
Afghanistan pea	0.6	2.6	1.8	3.2
Reference crop				
Nod$^-$ pea	0.7	1.3	0.7	0.5
Spring oilseed rape	1.3	3.1	1.4	0.5
Winter oilseed rape	2.7	4.5	2.8	-
Spring barley	3.8	7.1	1.5	5.3

Calculations using the average enrichment of references showed that 'Afghanistan' and 'Frisson' pea had fixed 2.7 and 12.4 g N m^{-2}, respectively, at maturity (Table 3). 'Frisson' pea took up almost the same amount of labelled and unlabelled soil N as did the oilseed rape references, but only half as much as was taken up by barley (Table 3). The symbiotic nitrogen fixation was al-

most the same using either the non-nodulating pea mutant, spring barley, or winter oilseed rape as the reference in the calculations (data not shown). At all harvests the %Ndfa was significantly lower when spring oilseed rape was used as reference, because of the lower ^{15}N enrichment (Table 2).

Table 2. ^{15}N enrichment with and without correction for seed-borne N in above-ground plant parts from Exp. 1.

Crop	Weeks after emergence				
	5	8	14		
			Straw	Seed	Biomass
	atom % ^{15}N excess without correction				
N_2 fixing crop					
Frisson pea	0.114	0.043	0.064	0.039	0.044
Afghanistan pea	0.183	0.173	0.118	0.076	0.088
Reference crops					
Nod$^-$ pea	0.188	0.189	0.190	0.208	0.198
Spring oilseed rape	0.220	0.161	0.147	0.162	0.151
Winter oilseed rape	0.285	0.219	0.193	0.203	0.200
Spring barley	0.229	0.199	0.182	0.191	0.187
$\bar{X}$ (references)	0.231	0.192			0.187
	atom % ^{15}N excess corrected*				
N_2 fixing crops					
Frisson pea	0.135	0.044	0.067	0.039	0.045
Afghanistan pea	0.229	0.180	0.121	0.077	0.089
Reference crops					
Nod$^-$ pea	0.219	0.204	0.204	0.231	0.216
Spring oilseed rape	0.222	0.162	0.147	0.162	0.152
Winter oilseed rape	0.286	0.219	0.199	-	0.199
Spring barley	0.240	0.203	0.202	0.206	0.205
$\bar{X}$ (references)	0.242	0.197			0.193

* It was assumed that 50% of the seed-borne N was found in above-ground plant parts. Grams of seed-borne N sown per m^2 were: 0.5, 0.2, 0.25, 0.02, 0.02 and 0.3 for 'Frisson' pea, 'Afghanistan' pea, nod$^-$ pea, spring oilseed rape, winter oilseed rape and spring barley, respectively.

Table 3. Estimated contributions (g N m^{-2}) from soil, labelled plant material, symbiotic nitrogen fixation and seeds to total above-ground N of crops in Exp. 1.

Crop	Total N	Nitrogen derived from			
		Soil	Plant material	Fixation	Seeds
		5 weeks after emergence			
Frisson pea	1.6	0.4	0.2	0.7 (44)	0.3
Afghanistan pea	0.6	0.4	0.1	0.03 (5)	0.1
Nod⁻ pea	0.7	0.5	0.1	-	0.1
Spring oilseed rape	1.3	1.0	0.3	-	0.01
Winter oilseed rape	2.7	1.9	0.8	-	0.01
Spring barley	3.8	2.8	0.8	-	0.2
		8 weeks after emergence			
Frisson pea	12.8	2.1	0.5	9.9 (78)	0.3
Afghanistan pea	2.6	1.8	0.5	0.2 (9)	0.1
Nod⁻ pea	1.3	0.9	0.3	-	0.1
Spring oilseed rape	3.1	2.5	0.5	-	0.01
Winter oilseed rape	4.5	3.5	1.0	-	0.01
Spring barley	7.1	5.4	1.5	-	0.2
		14 weeks after emergence			
Frisson pea	16.3	2.8	0.8	12.4 (77)	0.3
Afghanistan pea	5.0	1.7	0.5	2.7 (54)	0.1
Nod⁻ pea	1.2	0.9	0.2	-	0.1
Spring oilseed rape	1.9	1.6	0.3	-	0.01
Winter oilseed rape	2.8	2.2	0.6	-	0.01
Spring barley	6.8	5.2	1.4	-	0.2

Values in parentheses are the per cent of N derived from nitrogen fixation.

3.2. Experiment 2

The results of Exp. 2 is shown in Table 4. The ^{15}N enrichment of total plant N in winter wheat and vetch was 0.047 and 0.003 atom % ^{15}N excess, respectively. At this low level of enrichment estimation of nitrogen fixtion may be associated with error due to lack of precision in determination of the $^{15}N/^{14}N$ ratios. The big difference in the soil nitrogen uptake by the two crops may be due to a very low uptake of inorganic N in vetch during the autumn. If the ^{15}N enrichment in the mineralized N further decreased from autumn to harvest of the crop, the nitrogen fixation estimate may also be erroneous, because the crops took up soil nitrogen with different enrichments.

Table 4. Dry matter production, N concentration and ^{15}N enrichment in straw and seed of crops from Exp. 2. Estimated contributions from soil, labelled plant material and fixation are shown for total crop nitrogen.

Parameter	Crop			
	Winter wheat		Winter vetch	
	Straw	Seed	Straw	Seed
Dry matter (g m^{-2})	526	438	354	175
% N in dry matter	0.32	1.52	1.52	4.67
atom % ^{15}N excess	0.043	0.049	0.007	0.003
Soil N (g N m^{-2})	7.8		0.7	
Labelled N (g N m^{-2})	0.5		0.04	
N_2 fixation (g N m^{-2})	-		12.7	

3.3. Experiment 3

Uninoculated white lupin became sparsely nodulated in Risø soil. The nodules were small and white, indicating ineffective symbiosis. The dry matter production and nitrogen accumulation were 21 and 57% lower, respectively, than in inoculated and well-nodulated lupins (Table 5).

In this experiment, which was carried out 3 years after adding the labelled plant material, the ^{15}N enrichment of the nitrogen in the seeds of the non-fixing reference (spring wheat) was 0.030 atom % ^{15}N excess, but only 0.007 in straw (Table 5). The reason for this large difference in the enrichment of wheat plant parts is unknown. No weighed atom % ^{15}N could be calculated because seeds were lost, due to a late harvest.

Table 5. Dry matter production, N-concentration and ^{15}N enrichment of crops from Exp. 3.

Crop Plant part	Dry matter (g m^{-2})	% N	atom % ^{15}N excess
Spring wheat			
Seed	163*	1.93	0.030
Straw	389	0.62	0.007
Root	64	0.84	0.014
Lupin, uninoculated			
Pod	218	2.34	0.025
Shoot	356	1.47	0.024
Root	60	0.72	0.018
Lupin, inoculated			
Pod	204	3.31	0.010
Shoot	524	3.15	0.005
Root	77	1.94	0.007

* Seeds lost due to late harvest.

Estimating nitrogen fixation in different plant parts of inoculated white lupins, using wheat plant parts as references, showed that 67, 30 and 46% of the nitrogen in pods, shoot and roots, respectively, were derived from fixation. The lower ^{15}N enrichment of wheat straw and roots compared to uninoculated lupins, indicated that spring wheat may have taken up more unlabelled soil nitrogen from deeper layers than the lupins.

3.4. Mineralization and recovery of labelled N

Three weeks after incorporation of the material the ^{15}N enrichment of soil inorganic N (4.7 mg N kg^{-1}) was found to 0.164 atom % ^{15}N excess. In April 1984, when Exp. 1 was sown, the enrichment of the inorganic N (5.3 mg N kg^{-1}) was 0.155 atom % ^{15}N excess. The amount of inorganic labelled N present in the top soil in April corresponded to 2.1% of the added labelled organic N.

Table 6. Estimated contributions from soil, labelled plant material, nitrogen fixation and seeds to total N of inoculated white lupin plant parts. Exp. 3.

Plant part	g N m^{-2} derived from				Total N
	Soil	Plant material	Fixation	Seed	
Pod	2.0	0.1	4.5 (67)	0.1	6.7
Shoot	11.3	0.2	5.0 (30)	0.1	16.6
Root	0.8	0.0	0.7 (46)	0.0	1.5
Total	14.1	0.3	10.2 (41)	0.2	24.8

Values in parentheses are % N derived from fixation.

The laboratory incubation experiment showed that 73% of the carbon and 26% of the nitrogen was mineralized after 94 days of incubation at 20°C (Fig. 1 and Table 7).

The top soil (0-20 cm) was removed from the plot after harvest of Exp. 3 and stored in outdoor containers. In December 1987 (4 years after addition of the material) ^{15}N analysis of whole soil showed that the ^{15}N enrichment was now 0.014 atom % ^{15}N excess.

In December 1987 the ^{15}N enrichment was also measured in some weeds (Senico-species and Agropyrum repens) growing in the containers. The average enrichment was found to be 0.037 atom % ^{15}N excess. By comparison with the total N enrichment, it can be seen that the labelled N apparently still is mineralized at a higher rate than the native soil organic nitrogen.

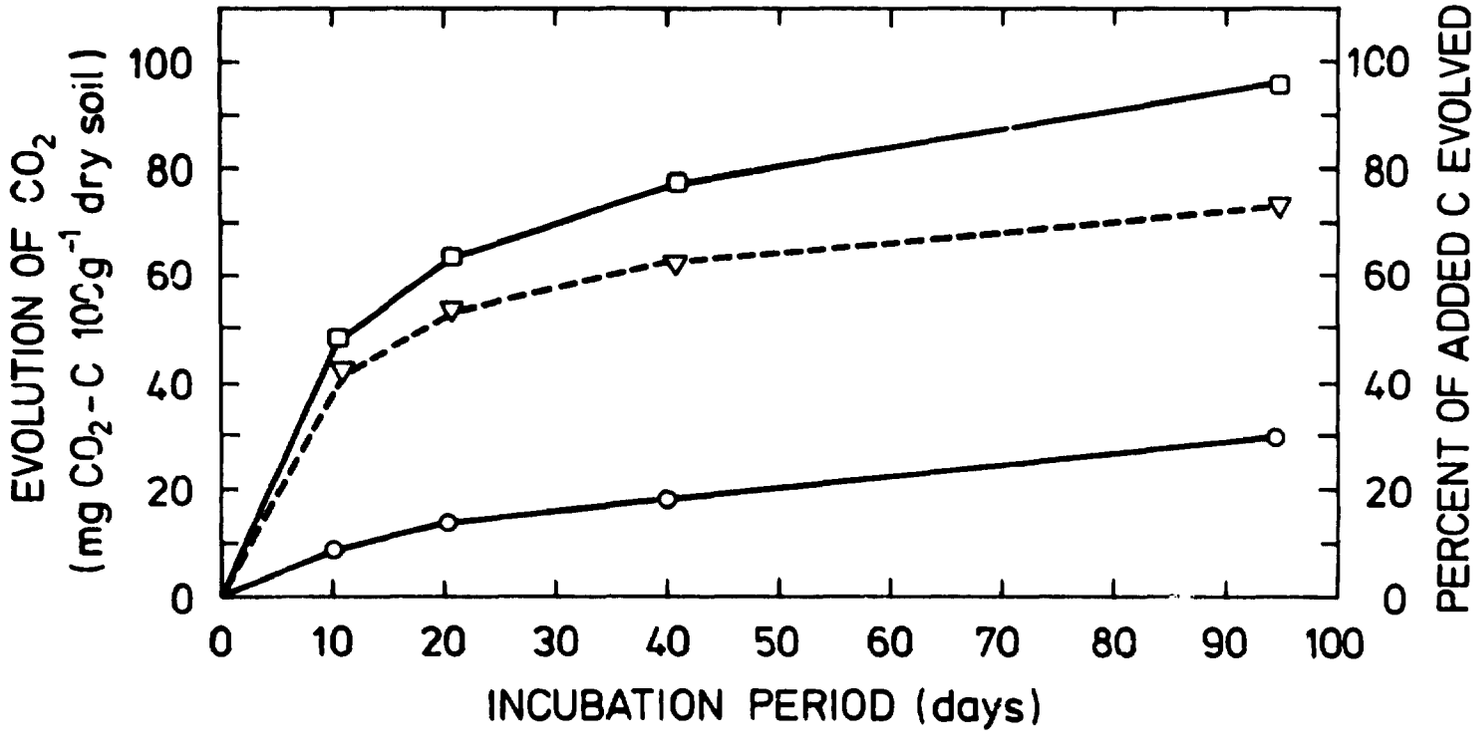

Fig. 1. Decomposition at 20°C of labelled plant material in the laboratory. CO_2 production from unamended soil (o), soil with plant material added (□). The broken line (▽) shows the shows the evolution of CO_2 from the amended soil as per cent of the amount of plant C added (93.2 mg C 100 g^{-1}).

Table 7. Inorganic N in soil with and without addition of labelled plant material after 94 incubation at 20°C in the laboratory.

	mg N kg^{-1} dry soil
N in plant material	43.8
KCl extractable at start	2.4 (0.4)*
KCl extractable after 94 d	
Unamended soil	22.6 (0.1)
Amended soil	34.1 (0.8)
Net mineralization	11.5

Values in parentheses are standard errors (n=4).

4. DISCUSSION

The two main advantages of adding ^{15}N labelled plant material or to immobilize $^{15}NH_4^+$-N with sugar and straw to the label soil nitrogen are 1) to obtain a more stable enrichment of the plant available soil N during growth and 2) and to avoid a reduction or stimulation in the nitrogen fixation due to addition of mineral N. When organic material is added to soil the microbial activity is increased if temperature and moisture are not limiting, and a high proportion of the added organic C is mineralized during the first months of decomposition (JENKINSON, 1984; SØRENSEN, 1981). In the present laboratory experiment 3/4 of the carbon was evolved as CO_2 after 3 month of incubation at 20°C. Depending on the nature of the material (age, C/N, lignin content, etc.) net immobilization or mineralization may occur during early decomposition of the plant material. Added organic N or N recently incorporated in the microbial biomass are normally mineralized with a higher rate than the majority of the native organic N. As the labelled N predominantly remain in recalcitrant remnants of the microbial biomass, it becomes gradually more resistant to

mineralization. The enrichment of mineralized N decreases thus with time. After some years the enrichment of the mineralized N remain on the same level, because the contribution of inorganic N from the labelled pool of organic N and from the native pool is proportionally of the same size.

In the present experiment the plant material was incorporated in November 1983 and allowed to decomposed during the late autumn and winter. Crops were established in April 1984. In the field some carbon was mineralized during the autumn, but the majority of the carbon was probably mineralized during the growing season.

In the laboratory incubation experiment 26% of the organic N added was estimated to have been mineralized after 94 days of incubation. In the field 2% of the labelled N was found in the top soil as inorganic N 3 weeks after incorporation. This labelled N was probably leached during the winter. In April 1984 2.1% of the labelled N added was found in the top soil as inorganic N, indicating that net mineralization of labelled organic N took place during the early spring.

The ^{15}N enrichment of the inorganic N in the top soil in December 1983 and April 1984 were 0.164 and 0.155 atom % ^{15}N, respectively. Plant emerged mid May and the nitrogen taken up in reference crops during the first 5 weeks of growth was on average 0.242 atom % ^{15}N excess. This indicate that the mineralization of labelled N was accelerated relative to the native N during May and June. The soil N taken up from 5 to 8 weeks after seedling emergence in the reference crops had on average an enrichment of 0.149 atom % ^{15}N excess. There was no net uptake of soil N from 8-14 weeks after seedling emergence.

WITTY (1983) found that the decline in ^{15}N enrichment of soil inorganic N during the period of plant uptake did fit an exponential curve. The decline in ^{15}N enrichment can be desribed mathematically as:

$$\text{atom \% } ^{15}N \text{ soil N} = a + be^{-Dt} \qquad \text{(WITTY, 1983)}$$

where a is the enrichment at time infinity, b is the enrichment at time zero, D is the decline constant, and t is the time in days. The faster the decline in ^{15}N enrichment during the growth season the higher is D, and the higher is the potential for error in estimating nitrogen fixation, if the legume and reference crop do not have the same patterns of N uptake (WITTY, 1983). Using the average enrichment of the nitrogen taken up in crops from 0 to 5 weeks and 5 to 8 weeks after seedling emergence, and assuming that the $\log_e$ to the atom % ^{15}N excess regressed against time were linear, a rough estimate of the decline constant was found to be 0.017. In the same year nitrogen fixation was measured in pea grown in a neighbouring field using spring barley as reference crop and addition of ^{15}N labelled nitrate at sowing (JENSEN, 1987). Nine harvests were taken during the growth season and the decline constant was calculated in the same way, using the average enrichment of the soil N taken up in barley in different periods as a measure of the plant available soil N enrichment. The decline constant was estimated to be 0.040. Barley harvested at almost the same times (5, 8 and 14 weeks after seedling emergence) had enrichments of 0.58, 0.50 and 0.37 atom % ^{15}N excess, respectively, compared to 0.23, 0.20 and 0.19 in Exp. 1. In comparison WITTY (1983) was able to reduce the decline constant from 0.030 to 0.013 by gypsum pelleting the ^{15}N labelled nitrate fertilizer. The results in Exp. 1 show that although not completely stable during the growth season, the stability of the enrichment was much higher when the labelled N was added in organic form as compared to adding inorganic ^{15}N at sowing.

In 1985 and 1986 the ^{15}N enrichment of the nitrogen in reference crops were 0.048 and 0.030 atom % ^{15}N excess, respectively. In 1987 the enrichment of N in weeds growing on the soil was 0.037. This indicate that the ^{15}N enrichment was becoming stable after 2 to 3 years. Since the ^{15}N enrichment of total soil N was only 0.014 atom % ^{15}N excess, a large part (about 60%) of the native organic N is presumably not or only very slightly susceptible to microbial mineralization. Stabilization of the enrichments in the mineralized inorganic N pool may occur before the enrichment of the inorganic pool is equal with the enrichment of the total soil N. The high ^{14}C age of some fractions of soil organic matter in-

dicate that a large part of the soil organic matter (and consequently organic soil N) is biologically inert (STOUT et al., 1981).

Early April 1984 the top soil contained approx. 5 mg inorganic N kg^{-1} soil, which is only about one third to one fourth the normal level of inorganic N present in Risø top soil at this time of the year. This low level may be due to stimulated microbial activity and N immobilization in the spring, caused by the added organic material. This reduced level of inorganic N may only have been temporary since estimated nitrogen fixation in 'Frisson' pea contributed with 77% of total N. This estimate is similar to the estimate for pea grown in the neighbouring field, where nitrogen fixation was estimated by adding labelled nitrate in the spring (JENSEN, 1987). If the mineral N content of the soil were depressed throughout the growth season, the proportion of N derived from air would probably have been higher in Exp. 1. Addition of high amount of carbon in plant material or sucrose may significantly reduce the inorganic N content, so it is important to await net mineralization to occur before the experiment is started. Otherwise the nitrogen fixation may be stimulated as observed by WITTY and RITZ (1984).

In Exp. 1 levels of ^{15}N enrichment in crops were suitable for estimating the nitrogen fixation. In the following years the levels were significantly lower, especially in the N_2 fixing crops, but detectable. The analytical precison in the determing the stable isotope ratios at these low levels may greatly influence the estimates of nitrogen fixation. Therefore it would been more optimal if the ^{15}N enrichment of the added plant material had been higher, in order to obtain more confident estimates of nitrogen fixation in the second, third and following years.

The level of enrichment in plant available and total soil N was 0.037 and 0.014, respectively, in 1987. This correspond to $\delta\ ^{15}N$ values of approx. 100 and 40 relative to atmospheric air. According to KOHL and SHEARER (1981) soil ^{15}N enrichment resulting in $\delta\ ^{15}N$ values of 100 to 200 in the plant material may be useful in other experiments. However, whether such soils can be utilized

for experimental purposes depend on the analytical precision in determing the $^{15}N/^{14}N$ ratios.

The recovery of labelled N in spring barley (Exp. 1), winter wheat (Exp. 2) and inoculated white lupins (Exp. 3) was 1.5, 0.5 and 0.3, respectively, which shows that a total of 2.3 g N m^{-2} or 23% of the labelled N added was recovered in the three crops. The total N concentration in soil was 1240 mg N kg^{-1} dry soil. Assuming a bulk density of 1.2 g cm^{-3} it can be estimated that 4.4 g labelled N was still in the top soil. The total recovery in plants and top soil was therefore 67%. Consequently, about 33% or 3.3 g N m^{-2} of the labelled N added could not be accounted for; it may be present in deeper soil layer, leached or denitrified after the 4 years.

Selecting the appropriate reference crop for a given legume may be difficult. It is often assumed that the non-nodulated plant, isoline, cultivar or mutant of a given legume may be the most suitable reference. It is not possible to grow non-nodulated peas in most European soils, and non-nodulating pea lines or mutants have not been available for use in field experimental scale. Seeds of the non-nodulating mutant F 4.58 of 'Frisson' pea was kindly made available for this experiment by Dr. Messager, Dijon, France. Non-nodulating mutants of the cultivar 'Finale' has now been made in our laboratory by Dr. K. Engvild (ENGVILD, 1987), and are available for small scale experiments.

The estimates of nitrogen fixation using either the non-nodulating pea mutant or barley gave similar results. This was because the ^{15}N enrichment of the plant N in the two crops were almost the same, despite the total N uptake in barley was more than 5 times higher than in the mutant. Spring oilseed rape had much lower ^{15}N enrichments than the other references, and consequently the estimates of N derived from fixation was much lower, when this crop was used. WITTY (1983) found that oilseed rape was the most suitable reference for pea, when he compared oilseed rape with grass and spring barley. However, GILLER and WITTY (1987) found that the ^{15}N enrichment of oilseed rape was much lower than in barley and uninoculated chickpea, when they used three differ-

ent methods of applying the labelled fertilizer N. Consequently, they also found significantly lower estimates of nitrogen fixation when using oilseed rape compared to using spring barley. The reason for this lower enrichment of oilseed rape compared to spring barley is unknown. In GILLER and WITTY (1987) experiments and in the present experiment barley took up 2 to 3 times the amount of N taken up by oilseed rape, so it is unlikely that the lower enrichment is due to a higher uptake of N from deeper soil layers by spring oilseed rape. It is also noteworthy that the ^{15}N enrichment in winter oilseed rape, which did not enter the reproductive growth stages, had the same enrichment as the barley crop.

Estimates of nitrogen fixation in 'Frisson' pea (77% of total N, 124 kg N ha^{-1}) were very close to previously reported estimates in pea (JENSEN, 1986a; 1986b; 1987). In 'Afghanistan' pea up to 8 weeks after seedling emergence less than 10% of total N was derived from fixation. During the reproductive growth stage the per cent of N derived from fixation was significantly increased (up to 54%) which is in agreement with another study on nitrogen fixation in this crop using the conventional method of applying the labelled N (E.S. JENSEN, unpublished).

In many experiments significant amount of ^{15}N labelled plant material may be thrown away after analysis. The material could be a valuable resource in other experiments, e.g. for measurement of dinitrogen fixation. The analytical precision in the determination of the $^{15}N/^{14}N$ ratios will be the limit for the kind of material which can be used in such experiments. In the present experiment the use of the plot for determination of nitrogen fixation in more than one year would have required a higher enrichment, with the analytical precision we had at that time in our laboratory. However, it is not often that the material is enriched more than 1%, since such high enrichments would be waste of ^{15}N, if the material is not to be used for other purposes.

In the present experiment a 20 m^2 plot was supplied with 200 g N in plant material with approx. 1 atom % ^{15}N excess. The cost of 1 g ^{15}N (10% enriched) is 75-100 U.S. dollars. The material used

in the present experiment therefore represented a value of 200-260 U.S. dollars.

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Use of ^{15}N enriched plant material for labelling of soil nitrogen in legume dinitrogen fixation experiments

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Abstract (Max. 2000 char.)

The soil nitrogen in a field plot was labelled with nitrogen-15 (^{15}N) by incorporating labelled plant material derived from previous experiments. The plot was used the following 3 years for determination of the amount of N_2 fixed by different leguminous plants.

The atom % ^{15}N excess in grains of cereals grown as reference crops was 0.20, 0.05 and 0.03 in the 3 years, respectively. In the first year the level of enrichment was adequate for estimating symbiotic nitrogen fixation. In the second and third year lack of precision in determination of the $^{15}N/^{14}N$ ratios of legume N, may have caused an error in estimates of nitrogen fixation.

About 23% of the labelled N was taken up by plants during the 3 years of cropping; after 4 years about 44% of the labelled N was found still to be present in the top soil.

The labelling of the soil nitrogen with organic bound ^{15}N, compared to adding mineral ^{15}N at sowing, is advantageous because the labelled N is released by mineralization so that the enrichment of the plant available soil N pool become more uniform during the growth season; and high levels of mineral N, which may depress the fixation process, is avoided.

Descriptors

CROPS; DECOMPOSITION; LEGUMINOSAE; MEASURING METHODS; NITROGEN FIXATION; NITROGEN 15; ORGANIC MATTER; SOILS; UPTAKE

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