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ORIGINAL PAPER

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Evaluation of a new approach to the nitrogen-15 isotope dilution technique, to estimate crop N uptake from organic residues in the field

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Abstract Field experiments were conducted to test a new approach for estimating crop N uptake from organic inputs. Soils were pre-labelled by applying ^{15}N fertiliser to soybean [*Glycine max* (L.) Merr] and common bean [*Phaseolus vulgaris* L.] crops. Additional ^{14}N plots which received unlabelled fertiliser were also established in the same way. The above-ground biomass from all four plots was harvested, stored and the plots left to over-winter. In the following summer ^{15}N-labelled residues were added to the unlabelled soils and unlabelled residues were added to the ^{15}N-labelled soils at a rate of 150 kg N ha^{-1}. All plots were cultivated and sown with maize (*Zea mays* L.). Control plots that did not receive residue application or any additional fertiliser N were also set up. The plots were harvested in late autumn. Maize derived 37 kg N ha^{-1} and 31 kg N ha^{-1} from the added soybean residues, estimated using the direct and indirect approach, respectively, in plots previously sown to soybean. N derived from the added common bean residues was estimated as 26 kg N ha^{-1} and 24 kg N ha^{-1} from the direct and indirect methods, respectively. In the plots previously sown to common bean, N derived from added soybean residue was 32 kg N ha^{-1} using the direct method and 33 kg N ha^{-1} using the indirect method. N derived from common bean residues was 22 kg N ha^{-1} and 21 kg N ha^{-1} estimated using the direct and indirect approaches, respectively. It was concluded that the modified indirect technique allows a reasonable estimate of N derived from residues and that this will enable further experiments to be conducted in which N derived from more complex matrices, such as manure or sewage sludge, can be determined.

Keywords Crop residues · Nitrogen · Organic residues

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Introduction

Organic materials are potentially important sources of N in crop production, especially for resource-poor farmers. In order to successfully manage organic materials as nutrient sources the parameters that determine the release and uptake of N by crops must be identified. A reliable method is therefore required to measure plant N uptake from organic materials.

The need for a field technique for measuring plant N uptake from complex organic residues has long been recognised. Stevenson et al. (1998) concluded that an accurate yield-independent method is required. Traditionally, total N difference methods have been used (Whitehead et al. 1989), but these are yield-dependent and therefore make it difficult to investigate parameters which affect N release. Measuring the N availability of crop residues to the subsequent crop has been estimated directly, by labelling plants with ^{15}N fertiliser then reapplying the residues to following crops (Yacob and Blair 1980; Jensen 1994). This is a relatively simple procedure and is later referred to as the "direct approach". Measuring N release from more complex materials such as manure or sewage sludge is more difficult. Experiments have been carried out in which manures are directly labelled with ^{15}N by feeding animals ^{15}N-labelled plant material (Peschke 1982; Kirchmann 1985; He et al. 1994; Sørensen et al. 1994a, 1994b; Sørensen and Jensen 1998). However, the direct labelling approach is expensive and logistically difficult, especially for field-based studies, and therefore a simpler and cheaper approach is required.

Prediction of the amount of N plants will take up from crop residues or manures is difficult, as 50% or more of the total N is in an organic form and the release of N will be dependent on the "quality" of the organic residue (Amberger et al. 1982; Flowers and Arnold 1993). Prediction of N release is complicated by the simultaneous mineralisation of organic residues, and immobilisation of inorganic N by the soil microbial biomass, following residue addition to the soil (Kirchmann

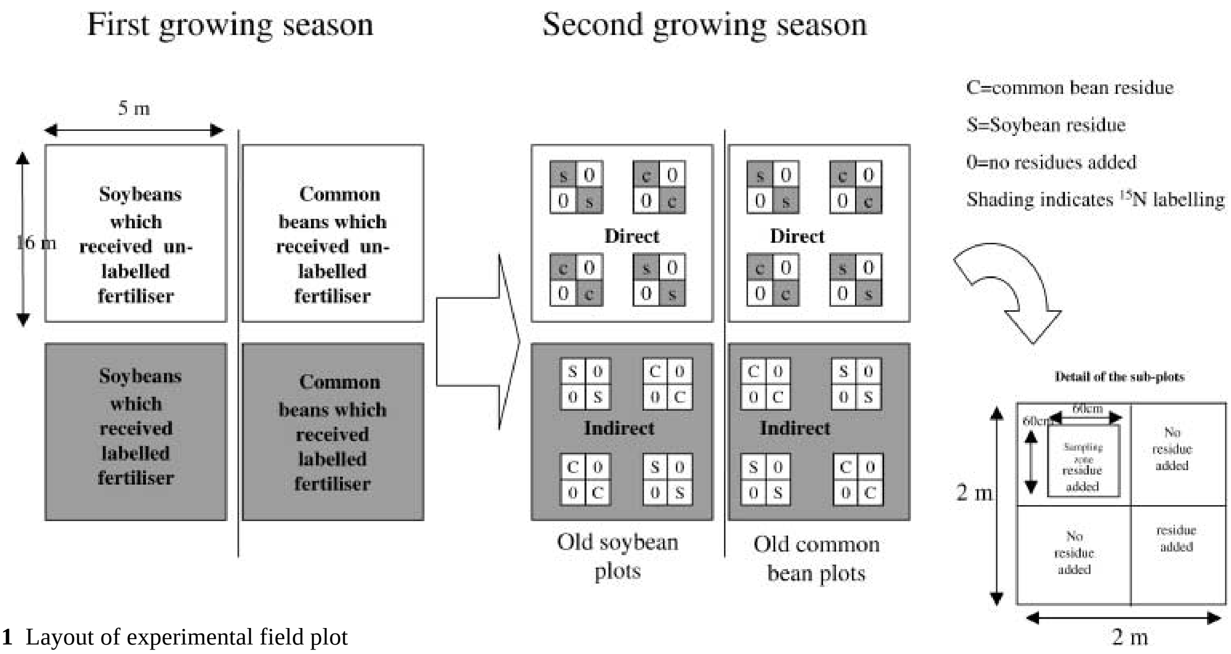

Fig. 1 Layout of experimental field plot

1985, 1991). This renders the conventional isotope dilution technique (simultaneous addition of residue and fertiliser) or *A* value techniques (Senaratne and Hardarson 1988; Stevenson et al. 1998) invalid for measuring plant N uptake from organic residues due to the problems associated with pool substitution (Hood et al. 1999). Pool substitution occurs when inorganic ^{15}N is immobilised and then replaced or substituted by ^{14}N from native soil N mineralisation (Hart et al. 1986). This results in a dilution of ^{15}N that is unrelated to ^{14}N release from the crop residues and would lead to an overestimate of N derived from residue. A detailed analysis of pool substitution is given by Hood et al. (2000).

The errors associated with pool substitution can be overcome by pre-labelling the soil with ^{15}N for a substantial period before the application of the residue of interest. The hypothesis is that when inorganic N is immobilised on addition of residues, inorganic N of a similar ^{15}N enrichment replaces it, allowing the isotope dilution technique to be used. This new approach was tested in the laboratory under controlled conditions and showed promising results (Hood et al. 2000). However, it needs to be tested in the field. The development of this method would allow plant N uptake from complex organic residues to be measured.

In this paper a field method is described in which the soil was pre-labelled with ^{15}N to a depth by root material of a preceding crop which had received labelled inorganic fertiliser. These plots were used in the subsequent year to test the new isotope dilution technique in the field (this technique is later referred to as the "indirect approach"). The new method was compared with the direct ^{15}N labelling technique for measuring crop N uptake from organic residues. The hypothesis being that if the new approach to the isotope dilution compares well with the direct approach, the new isotope dilution technique can be subsequently used to determine N release from more complex residues such as sewage sludge, manures, etc. Thus the design and timing of the experiment were primarily used to compare the two methods and not to gain agronomic data on N release from organic residues.

Materials and methods

The experiments were carried out at the FAO/IAEA Laboratories, Seibersdorf, Austria, The soil is a clay loam, with pH 8.2 (soil:water 1:2.5), total N 2.27 g kg^{-1} soil, extractable P 0.176 g kg^{-1} soil, extractable K g kg^{-1} soil, organic matter 62 g kg^{-1} soil (loss on ignition) or 40 g kg^{-1} soil (wet oxidation), and CEC 70.3 $cmol_c$ kg^{-1} soil. The growing season was summer, with average minimum air, maximum air and soil temperatures of 13°C and 24°C, and 22°C, respectively; total rainfall during the period was 154 mm and the plots also received irrigation on demand.

In the first year, ^{15}N-labelled and unlabelled, common bean (*Phaseolus vulgaris* L.) and soybean [*Glycine max* (L.) Merr] residues were produced on four large plots. These materials were used in the second year to test the direct and indirect techniques by applying labelled residues to the unlabelled soil (direct technique) and unlabelled residues to the labelled soil (indirect technique) (Fig. 1). Both residue types were tested on the plots previously sown to soybean and common bean (later referred to as the "old soybean" and "old common bean" plots, respectively).

In the first year of the experiment, common bean and soybean were sown on 10 June 1997 with an inter-row spacing of 38.5 cm and an intra-row spacing of 5 cm. Two plots of 5×16 m were set up for each crop, the labelled common bean plot received 30 kg N ha^{-1} and the labelled soybean 75 kg N ha^{-1} in the form of 10 atom % ^{15}N excess $(NH_4)_2SO_4$. The unlabelled plot received an equivalent amount of unlabelled $(NH_4)_2SO_4$. Both labelled and unlabelled fertiliser were applied as three applications, after germination (13 June 1997), during vegetative growth (10 July 1997) and during pod initiation (7 August 1997). Common bean above-ground biomass was harvested on 19 August 1997 and soybean on 22 August 1997. The crops were harvested prior to crop maturity as the aim of the experiment was not to gain agronomic data but to compare the direct and indirect techniques. The plots were maintained weed free over the winter of 1997–1998 and ploughed in the spring of 1998.

The labelled and unlabelled residues were individually harvested, separated into pods and shoots, dried at 70°C and ground to 4-mm-mesh size, and stored dry over winter. Sub-samples were ground to 200 µm for analysis. The N and C content and the ^{15}N enrichment of the material was determined using an IRMS Optima Micromass system (Micromass UK, Wythenshaw). Details of residues are given in Table 1. Only shoot material was used in the residue application experiment, because as stated above the aim was to compare the direct against the indirect technique.

As mentioned above, in the second year, crop residues were added to the soil at a rate of 150 kg N ha^{-1} as labelled or unlabelled common bean or soybean residues. In addition, control plots which received no residue were set up. The labelled soil plots received unlabelled residues. The unlabelled soil plots received labelled residues.

On each of the four main plots, four 2×2 m sub-plots were set up. These sub-plots were subdivided into 1-m^2 plots, two of which received residue and two no residue, giving a total of four 1-m^2 plots (Fig. 1). The plots were sown with maize (*Zea mays* L.) on 3 July 1998 with an inter-row spacing of 10 cm and an intra-row spacing of 10 cm. On 5 October 1998 an internal 60×60-cm square from each plot was harvested. Fresh matter yields were determined and plant material was chopped; sub-samples were dried, ground and analysed for N content and ^{15}N enrichment as described above.

Soil samples were taken with a 15-cm-long, 8-cm-diameter PVC core at application of the residues and at harvest. Inorganic N concentration and ^{15}N enrichment was determined on a 40-g fresh soil sample extracted in 200 ml of 1 M KCl. NH_4^+ and NO_3^- concentrations in the extracts were determined by flow injection analysis (Foss Tecator). The ^{15}N enrichment was determined by a modification (Hood et al. 2000) of the diffusion technique described by Brookes et al. (1989).

Calculations

Using the direct method the percentage N derived from residue (%Ndfr) is calculated using Eq. 1 (Hauck and Bremner 1976):

$$\%\text{Ndfr} = \left(\frac{\text{atom\% }^{15}\text{N excess of plant receiving labelled residues}}{\text{atom\% }^{15}\text{N excess of labelled residues}} \right) \times 100 \quad (1)$$

Using the soil indirect pre-labelling isotope dilution method, %Ndfr is calculated using Eq. 2 (McAuliffe et al. 1958):

$$\%\text{Ndfr} = \left(1 - \frac{\text{atom\% }^{15}\text{N excess}_{\text{treatment}}}{\text{atom\% }^{15}\text{N excess}_{\text{control}}} \right) \times 100 \quad (2)$$

Treatment is the plant grown with unlabelled residue amendment and control is the plant grown without residue.

The amount of N which is recovered from the residue (Ndfr) can be calculated using Eq. 3:

$$\%\text{N recovery from residue} = \frac{\text{Ndfr (kg)}}{\text{N added as residue (kg)}} \times 100 \quad (3)$$

Statistics

Results were analysed using one-way ANOVA with a $P<0.05$ indicating a significant difference. The packages Microsoft Excel and Jandel Scientific Sigma Stat were used.

Results

In the first growing season the total dry matter yield of the common bean plots was approximately 2 t ha^{-1} and for the soybean 3 t ha^{-1}.

In the old soybean plots at the end of the second cropping season, dry weight or total N content of the maize grown with the addition of soybean residues, or common bean, did not increase significantly ($P>0.05$) compared with the controls where no residue was added (Table 2). On the old common bean plots there was a significant increase ($P<0.05$) in the total dry weight and total N content of maize with the addition of the soybean residues compared with the controls which received no residues. Also, on the old common bean plots the addition of common bean residues caused a significant increase ($P<0.05$) in the dry weight but not in the total N content of the maize crop (Table 2). Where no residue was added to the soil, dry weight yield and total N yield of the

Table 1 Composition of residues

Residue	N concentration (g kg^{-1})	C:N ratio	^{15}N enrichment (atom % excess)
^{15}N-labelled common bean	28	15:1	0.840
Unlabelled common bean	26	16:1	0.003
^{15}N-labelled soybean	32	12:1	1.780
Unlabelled soybean	32	12:1	0.006

Table 2 Dry matter and total N content of shoots of maize grown in Seibersdorf soil amended with either soybean or common bean residues on the old common bean plots or soybean plots (average of direct and indirect plots). Data *in parentheses* are SEs (n=8)

Treatment	Dry matter (10^3 kg ha^{-1})	Total N (kg ha^{-1})	Dry matter (10^3 kg ha^{-1})	Total N (kg ha^{-1})
	Old common bean plots		Old soybean plots	
Soybean	16.9* (0.7)	146.6* (11.9)	12.9 (1.2)	145.4 (11.5)
No residue, Soybean control	12.6 (0.7)	109.4 (8.8)	15.6 (0.8)	160.2 (11.1)
Common bean	14.2* (1.2)	136.2* (11.8)	14.2 (1.2)	152.6 (10.2)
No residue, common bean control	12.6 (0.7)	104.3 (9.8)	14.8 (1.4)	147.0 (17.2)

*$P<0.05$ indicates a significant difference from the corresponding no residue control

Table 3 Estimates of percent N derived from residue (*% Ndfr*), amount of Ndfr in shoots of maize grown in Seibersdorf soil amended with either common bean or soybean residues on the old soybean plots or common bean plots. Data *in parentheses* are SEs (n=4)

Residue	%Ndfr		Ndfr (kg ha^{-1})		Ndfr calculated by N difference (kg ha^{-1})	
	Direct	Indirect	Direct	Indirect	Direct	Indirect
Old common bean plots						
Soybean	25.5 (4.5)	21.6 (4.1)	37.0 (5.2)	31.4 (4.5)	39	35
Common bean	17.1 (1.4)	15.4 (5.7)	26.1 (2.6)	23.3 (4.9)	28	35
Old soybean plots						
Soybean	21.9 (2.4)	22.8 (2.6)	32.1 (4.0)	33.4 (4.2)	13	–16
Common bean	16.0 (1.5)	15.3 (0.4)	21.8 (2.8)	20.8 (2.6)	19	–7

maize grown on the old soybean plots were significantly higher (P<0.05) than those of the maize grown on the old common bean plots (Table 2).

At planting (second season) there were no significant differences (P>0.05) in the extractable inorganic N between the four initial plots (approximately 8 kg inorganic N ha^{-1}). At harvest on the old soybean plots, there was no significant difference in total inorganic N between both residue treatments and the control (no residue treatment), which was approximately 10 kg inorganic N ha^{-1} in all treatments. However, on the old common bean plots there was a significant (P<0.05) increase in total inorganic N with both residue treatments (approximately 12 kg inorganic N ha^{-1} in both treatments) compared with the no residue control (8 kg inorganic N ha^{-1}).

N in the maize derived from soybean residues grown on the old soybean plots was 37 kg N ha^{-1} (25% Ndfr) estimated by the direct approach and 31 kg N ha^{-1} (22% Ndfr) by the indirect approach (Table 3). On the old common bean plots, N derived from the soybean residues was 32 kg N ha^{-1} (22% Ndfr) estimated by the direct approach and 33 kg N ha^{-1} (23% Ndfr) estimated by the indirect approach (Table 3). The values obtained using the direct and indirect approaches were not significantly different.

In the old soybean plots the amount of maize N derived from the common bean residues was 26 kg N ha^{-1} (17% Ndfr) estimated by the direct approach and 23 kg N ha^{-1} (15% Ndfr) estimated by the indirect approach (Table 3). In the old common bean plots the N derived from the common bean residues in the maize plants was 22 kg N ha^{-1} (16% Ndfr) and 21 kg N ha^{-1} (15% Ndfr) estimated by the direct and indirect approaches, respectively (Table 3). Again, the values obtained by the direct and indirect approaches were not significantly different.

The percentage of N added as common bean, recovered in the maize crop, was 15(±0.6)% and 14(±2.6)% of the total N added in the old soybean and common bean plots, respectively. The percentage N recoveries from the soybean residues were significantly higher, amounting to 24(±1.2)% in the soybean plot and 20(±2.8)% in the common bean plot.

Discussion

The values of Ndfr calculated using the indirect and direct techniques were similar, suggesting that the soil prelabelling may be suitable for use in studies to determine Ndfr from more complex residues. Ndfr from the soybean residue calculated directly and indirectly, respectively, was 37 kg N ha^{-1}and 31 kg N ha^{-1} on the old common bean plots and 32 kg N ha^{-1} and 33 kg N ha^{-1} on the old soybean plots. The values obtained using the N difference approach were in some cases negative, showing the problems of using a yield-dependent assessment. N derived from common bean residue (Ndfr) calculated directly and indirectly, respectively, was 26 kg N ha^{-1} (17%) and 23 kg N ha^{-1} (15%) on the old common bean plots, and 22 kg N ha^{-1} (16%) and 21 kg N ha^{-1} (15%) on the old soybean plots, respectively. Some negative values of Ndfr were obtained when they were calculated using the N difference approach (i.e. total N of crop in the residue-amended treatment, minus total N of the crop in the controls where no residues were applied). But generally values calculated using N difference were similar to those calculated using the isotopic methods on the old common bean plots, but not on the old soybean plots.

In the old soybean plots there was no significant increase in maize dry matter yield or total N content in both the treatments which received residues, compared to plots which received no residues, suggesting that these plots were not N limited. Both dry matter and N content of the maize were significantly higher in the old soybean plot controls (no residue) than in the old common bean plot controls (no residue). It can be calculated that the amount of available N is approximately 50 kg N ha^{-1} higher in the old soybean control plots compared to that in the old common bean control plots, suggesting a significant below-ground N input from the previous soybean crop. These values are comparable with those of Omay et al. (1998) who showed that N uptake in soybean-maize rotations was 70 kg N ha^{-1} greater than in continuous maize systems, with only 5 kg attributable to above-ground residue input, on a loam soil. This indicates that >60 kg N ha^{-1} came from the below-ground component. In the first year of this experiment above-ground N yield of the soybean plots was approximately 90 kg ha^{-1}. Rochester et al. (1998) showed that in soybean at maximum biomass, 40% of the N was in the roots, and this value corresponds to approximately 60 kg ha^{-1} below-ground N input.

Although there was no significant increase in the total N of the maize in the treatment with residue addition on the old soybean plots, there was a substantial proportion of the N in the maize derived from the residues, as shown by the direct data. This demonstrates the difficulty of using yield-dependent measurements. The N derived from the soybean residue was 37 kg N ha^{-1} (25% Ndfr) estimated using the direct approach and 31 kg N ha^{-1} (22% Ndfr) estimated using the indirect approach, and the difference between the two values was not significantly (P<0.05) different. The small difference may be attributable to the slight difference in C:N ratios of the labelled and unlabelled residues applied, the labelled residue having a slightly lower C:N ratio. In the old common bean plots values of N derived from the soybean residue were almost the same using both methods of estimation; 32 kg N ha^{-1} (22% Ndfr) and 33 kg N ha^{-1} (23% Ndfr) for direct and indirect methods, respectively, again showing that the direct and indirect methods gave similar results. However, these values are higher than those reported in the literature. Hesterman et al. (1987) found that maize derived 12% of its N from soybean residues 1 year after decomposition, and Omay et al. (1998) found that maize derived only 3–14% of its N from soybean residue. The higher values observed in the present study might be attributable to higher application rates of N as residue on a unit area basis or to the quality and finely ground nature of the residue applied.

N derived from common bean residue was significantly lower than the N derived from soybean residues on all plots. The common bean residue had a lower % N and higher C:N ratio compared with the soybean residue. However, it must be stressed that these studies were not intended to look at the agronomic benefits of the addition of residues. Many studies have shown that the C:N ratio is one of the major parameters affecting plant N uptake from residues when there are low total phenol concentrations (Berg and Staff 1981; Fox et al. 1990).

Stevenson et al. (1998) comparing A value techniques with the direct method observed that estimates using either approach were within 6 kg ha^{-1} for pea residues. However, it should be noted that the A value method was shown to be an unreliable method for estimating N derived from residues, especially when the C:N ratio of the residue is high (Hood et al. 1999). McDonagh et al. (1993) and Stevenson et al. (1998) observed that the results obtained using an indirect approach were more variable than with the direct approach. The greater variability of the indirect approach may be due to the fact that in the indirect technique, the variances of analysis are combined from both the control and the residue treatments. While in the direct approach the variance of analysis is based only on one set of data, i.e. the residue treatment.

The N recovery from the common bean residue was 14–15% and agrees with the values of 14–15% of the pea residue N recovered by the first crop in field studies reported by Jensen (1994). The N recovery from the soybean residues was slightly higher (20–24%), and this difference, as mentioned above, was almost certainly due to the higher N concentrations of the soybean residues [3.2% N, versus 2.6% N in the common bean, used in this study, and pea residues used by Jensen (1994)].

There have been some criticisms about the direct approach for measuring plant N uptake from organic residues. For example, Stevenson et al. (1998) suggested that quantities of soil N available to plants can be overestimated when labelled residues are added to soils, as unlabelled inorganic N can be immobilised and labelled N residue mineralised simultaneously, leading to an overestimate of the N contribution from the residue. However, the direct method is the most reliable available method for measuring plant N uptake from organic residues, and it is the best method with which to get results which are to be compared with those of the indirect method. The results of our experiments suggest that the modified soil labelling approach gives good agreement with the direct approach.

It can be concluded that the modified soil labelling approach allows reasonable measurement of N derived from residues, because it is assumed that the technique overcomes the problems associated with pool substitution. It is now hoped that this soil pre-labelling technique can be used to measure plant N uptake from more complex residues, such as sewage sludge or manures.

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