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Estimating crop N uptake from organic residues using a new approach to the ^{15}N isotope dilution technique

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Abstract

Experiments were conducted to test a new approach to the ^{15}N isotope dilution technique for estimating crop N uptake from organic inputs. Soils were pre-labelled with ^{15}N fertiliser and a carbon source. These were then incubated until there was stabilisation of the ^{15}N abundance of the inorganic N pool and resumption of inorganic N concentrations. Residues were then applied to the soils and planted with ryegrass (*Lolium perenne* L.) to determine the nitrogen derived from the residue (Ndfr) using the isotope dilution equations. This method was compared with the direct method, i.e. where ^{15}N-labelled residues were added to the soil and Ndfr in the ryegrass calculated directly. Estimates of percentage nitrogen derived from the residue (%Ndfr) alfalfa (*Medicago sativa* L.) in the ryegrass, were similar, 22 and 23% for the direct and soil pre-labelling methods, respectively, in the Wechsel sandy loam. Also, estimates of the %Ndfr from soybean (*Glycine max* (L.) Merr) residues in the Krumbach sandy loam were similar 34% (direct) and 36% (soil pre-labelling approach). However, in the Seibersdorf clay loam, the %Ndfr from soybean was 49% using the direct method and 61% using the soil pre-labelling method; yet Ndfr from common bean residue was 46% using the direct approach and 40% using the pre-labelling, not significantly different ($P > 0.05$). The soil pre-labelling approach appears to give realistic values for Ndfr. It was not possible to obtain an estimate of Ndfr using the soil pre-labelling method from the maize residues (*Zea mays* L.) in two of the soils, as there was no increase in the total N of the ryegrass over the growing period. This was probably due to microbial immobilisation of inorganic N, as a result of the wide C:N ratio of the residue added. The results suggest that the new soil pre-labelling method is feasible and that it is a potentially useful technique for measuring N release from a wide range or organic residues, but it requires further field-testing.

Introduction

Central to the development of sustainable agriculture is the adoption of farming practices that recycle organic resources such as crop residues, leguminous green manures, tree leaves and animal manures. To maximise the benefit of residues for crop use and efficient soil fertility management, it is essential to understand and quantify the transformations of N from these materials.

The research into plant N uptake from organic residues is limited due to methodological difficulties. Non-isotopic methods can provide useful agronomic information on the quantity of N becoming available to a crop: N uptake by a crop is measured in the presence and absence of added residues and the difference is attributed to N mineralised from the residue. There are, however, significant limitations to this approach. N release in practical situations is often

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rather small compared with total crop N uptake so the precision of measurement is poor (Powlson and Barraclough, 1993). Also, it is impossible to trace the flow of residue-derived N through soil pools without isotopic labelling and losses can only be determined if all possible pathways are measured (Powlson and Barraclough, 1993).

If a residue of interest can be uniformly labelled with ^{15}N, this provides an unambiguous method of tracing the fate of N from the residue and measuring the amount taken up by a crop (Ladd et al.,1981). This is the most direct method available but it also has limitations, both practical and theoretical due to uncertainties of interpretation in some situations (Watkins and Barraclough, 1996). Large quantities of expensive ^{15}N may be required to obtain sufficient labelled residue. Some residues such as manures or tree leaves are difficult to label, although labelled manures can be produced by feeding livestock with ^{15}N labelled plant material it is prohibitively expensive (He et al., 1994; Sørensen et al., 1994a,b; Sørensen and Jensen, 1998). Indirect ^{15}N isotope dilution techniques have been used (Kumarasinghe and Eskew, 1993; Senaratne and Hardarson, 1988; Stevenson et al., 1988), in which unlabelled residues and labelled inorganic N are added to soil simultaneously. However, Hood et al. (1999) showed that pool substitution as described by Jenkinson et al. (1985) and Hart et al. (1986) could lead to erroneous values for the quantity of N derived from mineralisation of residues. In this paper, we describe an approach in which soil was pre-labelled with ^{15}N a considerable time before adding unlabelled residues in order to minimise problems caused by pool substitution. It is hypothesised that by pre-labelling the soil with ^{15}N, the inorganic N pool and the incoming N from basal mineralisation are of a similar ^{15}N enrichment and are not altered by N immobilisation due to residue addition thus overcoming the problems associated with pool substitution. An analogous approach has been used in research to measure biological nitrogen fixation. Witty and Ritz (1984) introduced a technique in which the soil pool of plant-available N was pre-labelled with ^{15}N and fixation estimated from the degree of dilution.

The aim of the experiment was to test the concept of pre-labelling against the direct technique in the laboratory and to determine whether the indirect technique could be extended as a useful technique for measuring Ndfr from complex organic residues in the field using ^{15}N pre-labelling procedures.

Table 1. Description of the soils used

Soil property	Seibersdorf	Krumbach	Wechsel
Texture	Clay loam	Sandy loam	Sandy loam
pH (soil: water, 1: 2.5)	8.2	7.9	7.5
Total N (g kg^{-1} soil)	2.27	1.11	1.74
Extractable P (g kg^{-1} soil)	0.176	0.051	0.040
Extractable K (g kg^{-1} soil)	1.015	0.258	0.185
Organic matter[a] (g kg^{-1} soil)	62	29	78
Organic matter[b] (g kg^{-1} soil)	40	20	-
CEC (cmol$_c$ kg^{-1} soil)	70.3	20.9	37.5

[a] Loss on ignition.
[b] Wet oxidation.

Materials and methods

Pre-labelling of the soil was achieved by adding ^{15}N labelled ammonium together with a carbon substrate in order to immobilise the tracer. The soil was then incubated and the inorganic N pool was monitored to see when immobilisation was complete and whether the inorganic N levels had returned to a pre-addition level, secondly, to determine when the mineralised N was at a reasonably constant ^{15}N enrichment. The second stage of the experiment was initiated in treatments in which these criteria were best met in each of the soil types. All other pots were left to incubate further and those not used were eventually discarded. Residues were added to the soils, which were sown to ryegrass, no residue controls were also set up and ^{15}N and N content of the ryegrass determined. The new indirect pre-labelling approach was compared to the ^{15}N direct approach using a mirror image or cross labelling design.

Experimental conditions

The experiments were carried out under greenhouse conditions at the FAO/IAEA Agriculture and Biotechnology Laboratories, Seibersdorf, Austria. Mean day and night temperatures in the greenhouse were 28 °C and 20 °C, respectively. The light regime ranged from 220 to 860 μ moles m^{-2}s^{-1} for a 12 h photoperiod and the relative humidity varied between 60 and 70% (day and night amplitude).

Pre-labelling of soil

For each soil type, Seibersdorf, Krumbach and Wechsel (characteristics in Table 1), sieved soil (26 kg dry weight equivalent) was mixed 1:1 in an industrial cement mixer with quartz sand, after mixing 20 mg P kg^{-1}, 50 mg K kg^{-1} as $KH_2PO_4$and KCl and 70 mg N kg^{-1}soil as $(NH_4)_2SO_4$. Half the soil-sand mixture (26 kg) was labelled with enriched (50 atom % ^{15}N excess) $(NH_4)_2SO_4$ and half with the same amount of $(NH_4)_2SO_4$ at natural abundance. The ammonium solution was sprayed with a hand held sprayer evenly over the soil and mixed thoroughly. Care was taken to prevent cross contamination of ^{15}N. Four different C:N ratios were established using two sources of carbon: 6:1 (cellulose), 12:1 (cellulose), 24:1 (cellulose), 24:1 (straw) and 36:1 (straw), in order to determine the optimal level for pre-labelling. This resulted in a total of 15 paired treatments, three soil types, five carbon treatments, with unlabelled or labelled fertiliser added. Cellulose was initially selected as the C source, but wheat (*Triticum aestivum*) straw was also used as a cheaper alternative. The soils were mixed with additions, then divided into 8 kg (dry weight equivalent) batches and weighed into free draining plastic buckets. These were watered to approximately field capacity (10–30 kPa tension) with deionised water, placed in the greenhouse and watered every other day with deionised water. Each of the ^{15}N labelled replicate pots was sampled at 0, 7, 28, 42, 56, 84 and 105 days after mixing. An additional sampling was also made on the day prior to residue addition. Sampling was done by taking a 10 cm long, 1.5 cm diameter cylindrical soil core. Soil moisture content, KCl-extractable ammonium and nitrate, and the respective ^{15}N abundance of the inorganic nitrogen was determined.

Production of crop residues

The labelled and unlabelled alfalfa (*Medicago sativa* L.) residues used were grown in the greenhouse in semi-hydroponic culture in Perlite, supplemented with ^{15}N labelled or unlabelled Long Ashton nutrient solution (Hewitt, 1966) (100 ml per 2 l tray daily). The alfalfa shoots were harvested after 40 days.

Labelled and unlabelled maize, soybean and common bean were grown in the field on Seibersdorf soil at the FAO/IAEA Agriculture and Biotechnology Laboratories. The labelled maize plots received 5 atom % ^{15}N excess ammonium sulphate solution and the unlabelled plots received normal fertiliser at natural abundance. Each plot received a total of 200 kg N ha^{-1} in split applications. Common beans received 10 atom % ^{15}N excess ammonium sulphate and unlabelled fertiliser at a rate of 40 kg N ha^{-1}. Soybean plots received 100 kg N ha^{-1} at 10 atom % ^{15}N excess and unlabelled fertiliser in split applications. The numerous split applications of fertiliser were intended to achieve material with uniform ^{15}N distribution. The composition of the resulting residues, which were subsequently added to the soils, are given in Table 2.

Table 2. Compostion and amount of the residues added

Residue	N conc. (g kg^{-1})	C: N ratio	^{15}N enrichment (atom % excess)	Amount added (g kg^{-1})
^{15}N labelled alfalfa	48	9:1	2.649	2.1
Unlabelled alfalfa	44	9:1	0.010	2.1
^{15}N labelled maize	7	57:1	2.731	14.5
Unlabelled maize	7	57:1	0.015	14.5
^{15}N labelled common bean	28	15:1	0.840	3.5
Unlabelled common bean	26	16:1	0.003	3.5
^{15}N labelled soybean	32	12:1	1.780	3.1
Unlabelled soybean	32	12:1	0.006	3.1

Residue application

Experiment 1

In the Wechsel (24:1) soil, after 126 days of incubation, the second phase of the experiment was initiated, to determine the nitrogen recovery from two residue types, maize and alfalfa. ^{15}N labelled residues were added to unlabelled soil, giving the direct estimation of Ndfr and unlabelled (^{14}N) residues were added to the labelled soil treatment giving an estimation of Ndfr using the isotope dilution (soil pre-labelling) approach. Maize and alfalfa were added at a rate of 100 mg N kg^{-1} soil, 14.5 and 2.1 g dry matter kg^{-1} soil, respectively (Table 2). A zero residue treatment was also set up.

The residues were mixed into the soil using a cement mixer. Sand washing of the cement mixer between each mix ensured minimal ^{15}N cross contamination. Six replicates (1 kg dry weight equivalent of soil) of each treatment were weighed into plastic pots and sown with 2 g of perennial ryegrass seed (*Lolium perenne* L.*)*. The pots were watered to approximately field capacity (10–30 kPa tension) with deionised water and placed in the greenhouse and watered every other day with deionised water to maintain constant moisture content. The ryegrass was harvested by cutting the grass 4 cm above the soil level 35 and 69 days after sowing.

Experiment 2

After 241 days of incubation of the Krumbach (6:1) soil, nitrogen derived from maize and soybean residues using the two methods was compared. Each treatment received 100 mg N kg^{-1} soil (Table 2) in the form of labelled or unlabelled residues and in addition, no residue treatments were also set up, giving a total of six treatments with six replicates. The experimental procedure was identical to the above, although only 0.5 g of perennial ryegrass seed was sown and there was only one harvest at 35 days after sowing.

Experiment 3

After 247 days of incubation, the Seibersdorf (12:1) soil was amended with 100 mg N kg^{-1} soil of labelled and unlabelled soybean or common bean residue 3.1 and 3.5 g dry matter kg^{-1} soil, respectively (Table 2). Treatments without residues were also set up. Again, the experimental set up was identical to that of the Wechsel soil, i.e. six treatments, six replicates, although only 1 g of perennial ryegrass seed was sown and only one harvest was taken at 35 days after sowing.

Analysis

Forty grams of fresh soil was sub-sampled from each core and shaken with 200 ml of 1 M KCl for 1 h before being filtered through glass fibre filter paper (GF/A Whatman). Soil moisture content (105 °C) was determined simultaneously. Ammonium and nitrate concentrations in the extracts were determined by flow injection analysis (Foss Tecator Ltd.). ^{15}N enrichment was determined by a modification of the diffusion technique described by Brookes et al. (1989). Fifty ml of the KCl extract was weighed into a 200 ml plastic vessel, two glass beads (0.5 cm diameter) and approximately 0.2 g of MgO were added and the vessel was closed. The ammonia evolved was collected on a 5 mm diameter glass fibre filter disc (Whatman GF/D) and acidified with 10 μl of 2.5 M potassium hydrogen sulphate. The filter disc was suspended from a bent stainless steel syringe needle attached to the lid by 'Blu-tack' adhesive (Bostik Ltd, Leicester, UK). The pots were stored in the dark at room temperature-pressure for 5 days, after which the disc was removed and dried in a desiccator containing dry silica gel and a beaker containing 20 ml of concentrated sulphuric acid. The vessels were left open for 24 h to remove any trace ammonium and subsequently prepared for nitrate determination. Devarda's alloy (0.2 g) was added and the vessel closed with a new lid with an acidified disc attached. These were left to diffuse for a further 5 days and the discs removed and dried as above. Check standards to determine recovery and cross over were also included. The dried filter discs were analysed for ^{15}N by an IRMS Optima Micromass system (Micromass UK, Wythenshaw) linked to a Carlo Erba Strumentazione nitrogen-carbon analyser 1500 combustion unit (Milan, Italy).

All harvested plant material and residues were dried at 70 °C to constant weight and ground to 200 μm. The added residues were analysed for total N and C with a carbon-nitrogen analyser and IRMS as described above.

Determination of seed N contribution to the shoots and roots

In the above experiments, it was necessary to determine the seed N input to shoot N due to the relatively high seeding rate. One gram of ryegrass seeds was sown into one kg quartz sand in pots (eight replicates) and watered daily with 5 atom % ^{15}N excess Long Ashton solution and grown in the greenhouse as described above. After 35 days, shoots were harvested from all pots, and from four of the pots the roots were also harvested. The remaining four were left to grow for a further 35 days and then roots and shoots were sampled.

Calculations

Using the direct method, the percentage nitrogen derived from residue (%Ndfr) is calculated: (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 pre-labelling isotope dilution method %Ndfr is calculated: (McAuliffe et al., 1958):

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

Where treatment = plant grown with residue amendment. Control = plant grown without residue.

A correction of the ^{15}N enrichment to account for seed N or initial plant N is necessary when initial N is a significant proportion of the final total N when using the pre-labelling approach, as was the case in these experiments. The correction is calculated as in Equation (3) and the corrected values, for both the 'treatment' and 'control' (no residues added) are used in Equation (2) (McNeill et al., 1994).

$$\text{Corrected } ^{15}\text{N} = \left(\frac{\left(^{t=t}\text{N} \times ^{t=t}\text{N}^*\right) - \left(^{t=0}\text{N} \times ^{t=0}\text{N}^*\right)}{^{t=t}\text{N} - ^{t=0}\text{N}}\right) \quad (3)$$

Where $^{t=0}$N and $^{t=0}$N* equal total N and ^{15}N enrichment (atom % ^{15}N excess) of seed in this case, $^{t=t}$N and $^{t=t}$N* equal total N and ^{15}N enrichment at harvest. The ^{15}N enrichment of the seed N was 0.0 atom % ^{15}N excess (natural abundance).

Nitrogen derived from residue expressed as an amount can be calculated:

$$\text{Ndfr (mg)} = \frac{\%\text{Ndfr}}{100} \times \text{ total N (mg)} \quad (4)$$

The amount of nitrogen which is recovered from the residue can be calculated:

$$\%\text{ N recovery from residue} = \frac{\text{Ndfr (mg)}}{\text{N added as residue (mg)}} \times 100 \quad (5)$$

Throughout this paper, data referring to the uptake of N by ryegrass is expressed in two ways, first the amount of N derived a given residue, expressed as mg pot^{-1} (Equation (4)). Second, as the percentage of the total N in the ryegrass that is derived from residue, %Ndfr (Equation (2)). The second value varies according to the quantity of N derived from other sources but the first value does not.

Statistics

All 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

Soil pre-labelling

Initial nitrate concentrations of the Wechsel, Krumbach and Seibersdorf soils prior to mixing were 205, 41 and 25 mg N kg^{-1} soil, respectively, with ammonium concentration in all soils less than 2 mg N kg^{-1} soil. Nitrification rates were apparently extremely high in all the incubation treatments. Although ammonium was added, the dominant form of inorganic N was nitrate; ammonium concentrations were less than 2 mg kg^{-1}soil 7 days after incubation and remained undetectable or negligible throughout the incubation period. In the Seibersdorf and Krumbach soil straw 36:1, and Seibersdorf soil straw 24:1 treatment, the nitrate concentration declined over the initial 28 days of incubation and remained less than 2.0 mg N kg^{-1}soil throughout. In the rest of the incubation treatments, there was an initial immobilisation of inorganic N over the 0 – 42 day period reaching a minimum around 42 days. This was followed by a general increase in nitrate concentration, although this was erratic and variable, and may have been due to the watering regime or the sampling procedure. In the Wechsel and Seiberdorf soil, nitrate ranged from 100 to 200 mg N kg^{-1} soil; in the Krumbach soil concentrations were lower than 100 mg N kg^{-1} soil.

In all the incubation treatments, the decline in the ^{15}N enrichment of the ammonium pool was characteristically an exponential decay followed by a linear phase (with an average r^2 of 0.96), the ammonium concentration remained negligible after 14 days in all treatments, making accurate determination of ^{15}N concentrations difficult. In all the soils, the ^{15}N enrichment of the nitrate pool rose in the initial days as the labelled ammonium was rapidly nitrified and then stabilised (Figure 1). The ^{15}N enrichment of the 6:1 treatment was consistently higher in all soil types.

Three incubation treatments were selected for comparing the direct and pre-labelling techniques. The selection criteria were that the ^{15}N enrichment of the extractable inorganic NO_3 pool had stabilised (Figure 1) and the inorganic N levels had returned to approximately initial concentrations (Figure 2). These were the Wechsel 24:1 (straw), Seibersdorf 12:1 (cellulose) and Krumbach 6:1 (cellulose) treatments.

Seed N contribution to shoots

Fifty percent of the seed N was in the shoot and fifty percent in the root 35 days after planting (four replic-

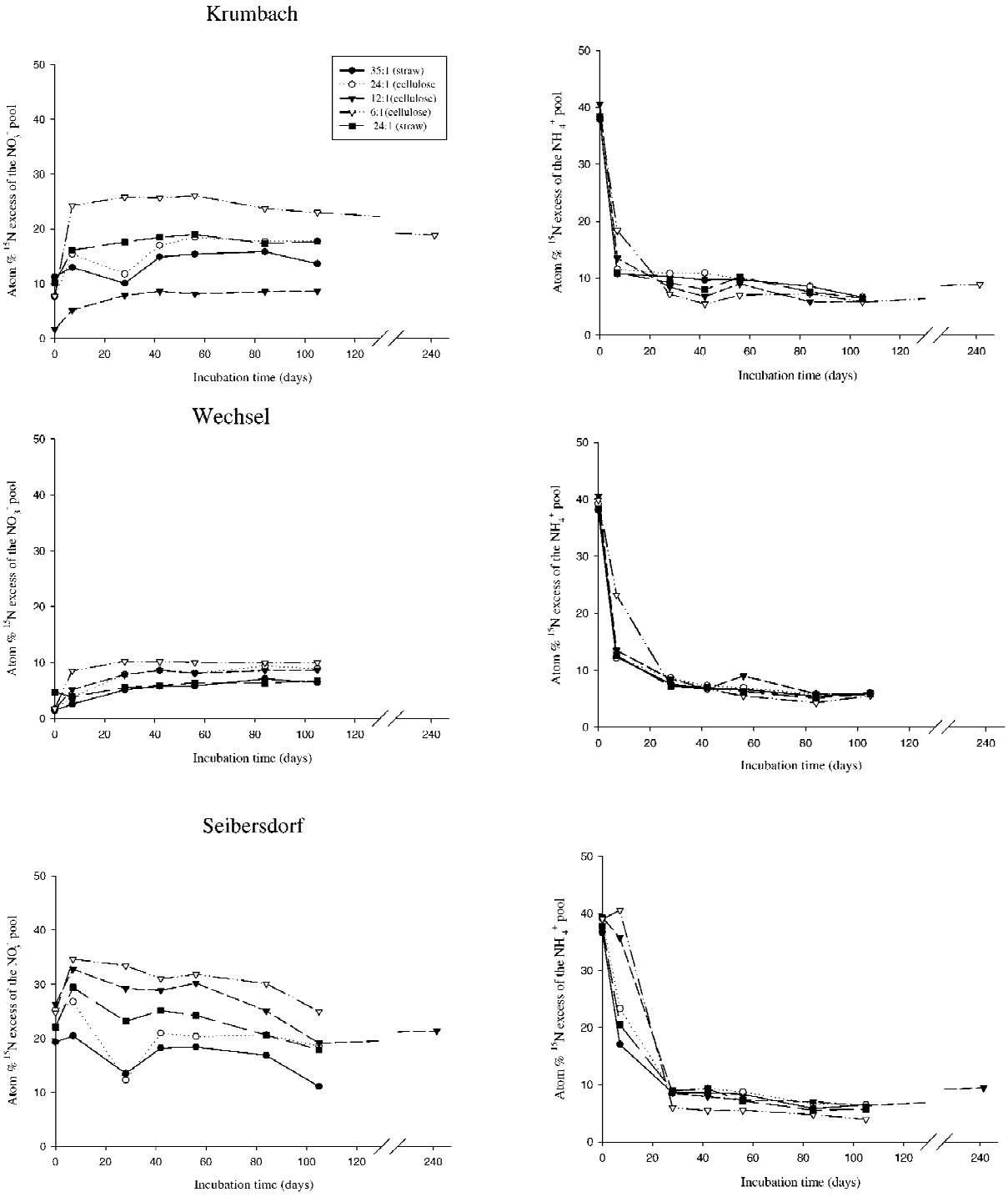

Figure 1. Average ^{15}N enrichment of the ammonium and nitrate pools in the incubated soils over time, n=3.

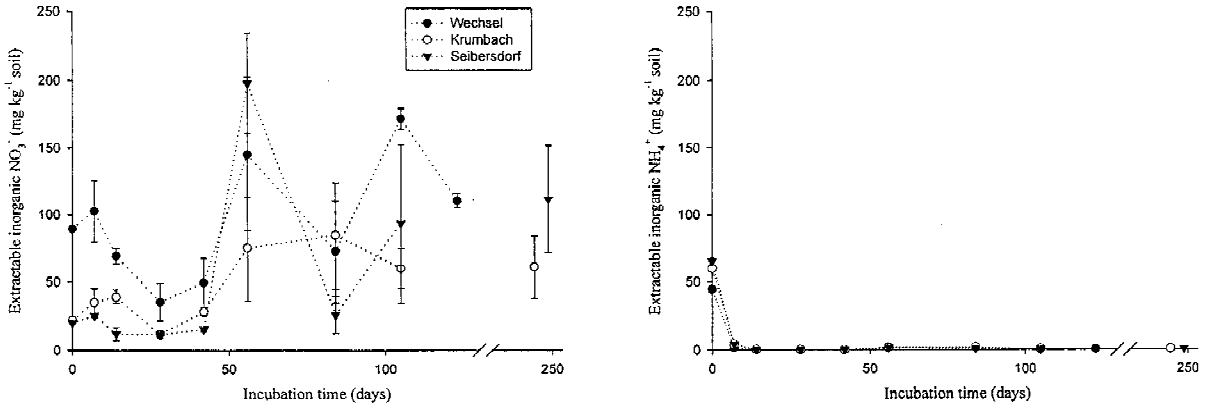

Figure 2. Concentration of inorganic N ammonium and nitrate in the incubated soils over time. Bars represent + and – standard error, n=3.

ates standard error 1.31%) showing good agreement with Jensen et al. (1985). Only 10% of the seed N was in the shoot 35 days after the second cut (four replicates standard error 1.6%).

Experiment 1: Wechsel soil

At the first and second harvest, 35 and 69 days after sowing, there were no significant differences ($P > 0.05$) either in the dry weight or total plant N between the labelled or unlabelled treatments, i.e. matched pairs (Table 3). Average total N in the alfalfa residue treatment was significantly higher than in the no residue treatments. Average total N and dry weight of the ryegrass from the maize residue treatment was significantly lower than both the no residue treatments and the alfalfa residue treatment.

Nitrogen derived from alfalfa residue was 20.7 mg pot^{-1} estimated using the direct method and 23.1 mg pot^{-1} estimated using soil pre-labelling method, assuming 50% initial seed N in the shoots. At the second harvest in the direct alfalfa treatment the %Ndfr did not change significantly from the first harvest, but the amount of N derived from alfalfa residue decreased from 20.7 mg at the first harvest to 4.3 mg pot^{-1} between the first and the second harvest. This was not significantly different from Ndfr calculated using the pre-labelling approach 4.6 mg pot^{-1} assuming 10% of the seed N was in the shoots (Table 4).

Using the direct approach at the first harvest, the nitrogen derived from the maize residue in the ryegrass was 0.8 mg pot^{-1}. However, it was impossible to estimate Ndfr using the pre-labelling approach, due to the high initial seed N compared with the lower total N content at harvest (Table 3). At the second harvest, Ndfr calculated using the direct approach was 2.3 mg pot^{-1}, this was not significantly different from the value of 2.4 mg pot^{-1} calculated using the pre-labelling approach (Table 4), again assuming 10% initial seed N in the shoots at the time of harvest.

Experiment 2: Seibersdorf soil

Total N and dry weight of the ryegrass was significantly ($P > 0.05$) higher in the pre-labelling treatment than the direct soybean residue treatment, and both values were significantly higher than in the no residue/^{14}N-soil treatment (Table 5). There were no significant differences between the dry weight and total N of the ryegrass in the direct and indirect common bean residue treatments, and they were significantly higher than the no residue/^{14}N-soil treatment. The total N and dry weight of the ryegrass in the ^{15}N labelled soil/no residue treatment was significantly higher than all treatments. This was checked and found to be inexplicable (Table 5).

Nitrogen derived from soybean residues calculated using the direct approach was 7.8 mg pot^{-1} and was significantly different ($P > 0.05$) from the 10.9 mg pot^{-1} estimated using the pre-labelling approach, again assuming 50% seed nitrogen (Table 6). In the common bean treatment, the Ndfr calculated using the direct and pre-labelling approach assuming 50% seed N, was not significantly different ($P > 0.05$).

Experiment 3: Krumbach soil

The dry weights and total N per pot in both residue treatments were significantly lower than in the control (Table 7). Dry weight and total N in the ^{15}N labelled soybean direct treatment were significantly higher than in the ^{14}N-soybean residue treatment. Nitrogen derived from ^{15}N-labelled soybean residues was 7.8

Table 3. Dry matter, total N and ^{15}N enrichement in shoots of ryegrass grown in Wechsel soil amended with either alfalfa or maize residues

Treatment	Dry matter (g pot^{-1})		Total N (mg pot^{-1})		^{15}N enrichment (atom% excess)	
	1st harvest	2nd harvest	1st harvest	2nd harvest	1st harvest	2nd harvest
Direct Alfalfa	3.02 (0.16)	1.59 (0.04)	94.1 (4.2)	20.6 (1.1)	0.583 (0.019)	0.558 (0.015)
Indirect Alfalfa	3.02 (0.11)	1.60 (0.08)	103.9 (4.0)	21.1 (1.4)	9.557 (0.216)	8.539 (0.131)
Direct Maize	0.70 (0.44)	0.95 (0.26)	12.2 (0.6)	14.1 (0.7)	0.186 (0.023)	0.435 (0.011)
Indirect Maize	0.70 (0.02)	0.84 (0.07)	13.5 (1.4)	12.2 (1.2)	2.183 (0.415)	7.387 (0.755)
Direct No residues	2.90 (0.22)	1.62 (0.16)	83.8 (2.8)	18.5 (0.61)	0.021 (0.000)	0.033 (0.000)
Indirect No residues	3.05 (0.19)	1.61 (0.10)	84.4 (1.7)	16.8 (0.4)	11.736 (0.166)	10.447 (0.141)

Data in parentheses are standard errors (n=6).

Table 4. Estimates (uncorrected for seed N) of nitrogen derived from residues (Ndfr) in shoots of ryegrass grown in Wechsel soil amended with either alfalfa or maize residues

Residue	%Ndfr direct		Ndfr direct (mg pot^{-1})		%Ndfr indirect		Ndfr indirect (mg pot^{-1})	
	1st harvest	2nd harvest	1st harvest	2nd harvest	1st harvest	2nd harvest	1st harvest	2nd harvest
Alfalfa	22.0 (0.7)	21.1 (0.5)	20.7 (1.3)	4.3 (0.3)	22.3 (2.5)	22.0 (1.0)	23.1 (0.85)	4.6 (0.3)
Maize	6.8 (0.8)	15.9 (0.4)	0.8 (0.1)	2.3 (0.1)	NA	19.9 (2.4)	NA	2.4 (0.2)

Data in parentheses are standard errors (n=6), NA = not applicable.

mg pot^{-1} compared with 6.2 mg pot^{-1} estimated using the pre-labelling approach corrected for seed N, and these values were not significantly different ($P >$ 0.05) (Table 8).

In the maize residue treatment, again it was not possible to obtain an estimate of Ndfr using the pre-labelling approach, but the Ndfr using the direct approach was low (0.04 mg N pot^{-1}, 3.3%Ndfr).

N recovery from residue was calculated using Equation (5) and the direct data from all experiments for the first harvest. The N recovery from alfalfa residue in the Wechsel soil was the highest (20.7%) (data not shown). In the Krumbach and Seibersdorf soils, the recovery of soybean residue N was similar 7.7 and 7.8%, respectively. The recovery of N from the maize was low 0.04 and 0.8% in both the Krumbach and Wechsel soils, respectively. N recovery from common bean residue 5.9% in Seibersdorf soil was significantly less than from soybean or alfalfa.

Table 5. Dry matter, total N and ^{15}N enrichment in shoots of ryegrass grown in Seibersdorf soil amended with either soybean or common bean residues

Treatment	Dry matter (g pot^{-1})	Total N (mg pot^{-1})	Atom %^{15}N excess
Direct Soybean	1.00 (0.03)	15.8 (2.4)	0.878 (0.019)
Indirect Soybean	1.17 (0.07)	18.0 (1.0)	5.179 (0.384)
Direct Common bean	0.77 (0.04)	12.8 (0.5)	0.384 (0.008)
Indirect Common bean	0.89 (0.05)	13.8 (0.69)	5.220 (0.550)
Direct No residues	0.85 (0.03)	10.3 (0.6)	0.169 (0.024)
Indirect No residues	1.81 (0.05)	28.3 (0.72)	18.300 (0.176)

Data in parentheses are standard errors (n=6).

Table 6. Estimates (corrected for seed N) of nitrogen derived from residues (Ndfr) in shoots of ryegrass grown in Seibersdorf soil amended with either common bean or soybean residues

Residue	%Ndfr		Ndfr (mg pot^{-1})	
	Direct	Indirect	Direct	Indirect
Soybean	49.3 (1.0)	60.6 (2.0)	7.8 (0.4)	10.9 (0.6)
Common bean	45.7 (1.0)	39.7 (5.5)	5.9 (0.3)	5.5 (0.3)

Data in parentheses are standard errors (n=6).

Table 7. Dry matter, total N and ^{15}N enrichment in shoots of ryegrass grown in Krumbach soil amended with either maize or soybean residues

Treatment	Dry matter (g pot^{-1})	Total N (mg pot^{-1})	Atom %^{15}N excess
Direct Soybean	1.10 (0.04)	23.6 (1.7)	0.601 (0.032)
Indirect Soybean	0.89 (0.08)	17.2 (1.4)	11.831 (0.382)
Indirect maize	0.08 (0.02)	0.9 (0.2)	0.578 (0.082)
Direct maize	0.13 (0.02)	1.1 (0.2)	0.094 (0.071)
Direct No residues	1.44 (0.04)	28.0 (1.0)	0.022 (0.000)
Indirect No residues	1.29 (0.02)	23.3 (0.3)	20.694 (0.252)

Data in parentheses are standard errors (n=6).

Table 8. Estimates (corrected for seed N) of nitrogen derived from residues (Ndfr) in shoots of ryegrass grown in Krumbach soil amended with either maize or soybean residues

Residue	%Ndfr		Ndfr (mg pot^{-1})	
	Direct	Indirect	Direct	Indirect
Soybean	33.8 (1.8)	36.1 (0.9)	7.8 (0.2)	6.2 (0.6)
Maize	3.3 (0.2)	NA	0.04 (0.00)	NA

Data in parentheses are standard errors (n=6), NA = not applicable.

Discussion

Soil pre-labelling

The inorganic N and ^{15}N incubation data from all soil types suggested that the microbial biomass immobilised most of the added inorganic nitrogen, as previously demonstrated by Alexander (1977) and Seligman et al. (1986). The immobilised nitrogen was then mineralised over time to give a stable ^{15}N enrichment in the inorganic N pool as hypothesised and shown in Figure 1. It is interesting to note that the highest ^{15}N enrichment was consistently observed in the 6:1 C:N ratio treatment, demonstrating that incomplete immobilisation has a significant impact on the ^{15}N enrichment of the inorganic N pool and highlights the problems associated with adding residues and inorganic N simultaneously. It also suggests that pre-labelling should be carried out at higher C:N ratios. The data in Figures 1 and 2 suggests that there was initially rapid nitrification of the labelled ammonium in both the Seibersdorf and Krumbach soils, as shown by the high enrichment of the nitrate pool. By calculating the nitrate derived from fertiliser using

the ^{15}N data and inorganic N data, it was apparent that over 80% of the added ammonium had actually been immobilised in the Seibersdorf and Krumbach soils and over 90% in the Wechsel soil at 42 days (data from Figures 1 and 2). The ^{15}N enrichments of the grass grown in soils with no residues added were of the approximately the same enrichment as their respective nitrate pools suggesting that the N coming from mineralisation was of approximately the same enrichment as the nitrate pool, assuming active mineralisation.

The lower ^{15}N enrichment of the ammonium pool compared to the nitrate pool was probably due to the effect of initial rapid nitrification of highly labelled ammonium followed by incomplete immobilisation of the nitrate pool, resulting in a high residual enrichment of the nitrate pool. This highlights the importance of selecting the correct C:N ratio for pre-labelling to ensure complete immobilisation of inorganic N.

In retrospect, the ideal labelling strategy would have been to have complete immobilisation of the inorganic N pool and subsequent mineralisation, however this would have required an excessively long incubation period. The results suggest that for pre-labelling with a C:N ratio of around 24:1, is the best for short term experiments. This allows stabilisation over a period of 4–5 months at greenhouse temperatures. The C:N ratio of the pre-label should also take account of the initial inorganic N of the soil. It also suggests that other pre-labelling techniques should be tested.

Comparison of the pre-labelling (indirect) and direct techniques

It was impossible to calculate nitrogen derived from residue using the isotope dilution technique in the first harvest maize residue treatments. However, in the second harvest of the Wechsel soil Ndfr calculated using the isotope dilution approach was not significantly different from the Ndfr calculated using the direct approach (assuming 10% seed N). One of the problems with any technique to measure N release from organic residues is the simultaneous immobilisation and mineralisation of N. In this case, the immobilisation was significantly greater than the mineralisation of N leading to significantly lower N contents than the no N control and initial seed N.

In the Wechsel soil, alfalfa treatment the values of Ndfr calculated using the pre-labelling and direct approaches were not significantly different ($P > 0.05$) and were very close in both harvests, suggesting that the new approach to the isotope dilution technique was estimating Ndfr as well as the direct approach. In similar experiments using the same soil Hood et al.(1999) estimated %Ndfr from alfalfa as 34% using the conventional isotope dilution approach (i.e. simultaneous addition of residue and label) compared with 22% using the direct approach, suggesting that pre-labelling of the soil reduces the errors associated with pool substitution. The relatively low %Ndfr in the alfalfa treatment can be in part attributed to the high inorganic N status of the soil.

In the Seibersdorf soil, the estimates of Ndfr using the direct and pre-labbelling approaches were different. The common bean residues gave similar values using both approaches. In the soybean treatment, Ndfr calculated using the pre-labelling approach was significantly higher than that calculated using the direct approach. This may have been a result of the higher total N and better plant growth in the pre-labelling treatment or due to problems associated with incomplete immobilisation of the nitrate pool in the incubation period. The difference between direct and pre-labelling treatments implies they were not identical in all but the position of the ^{15}N label. Obtaining well matched cross-labelling pairs is central to the testing of the direct against the pre-labelling technique, every effort was made at all stages to ensure incubation and growing conditions were similar. However, small differences in residue quality, mixing, watering or placement of soils may have led to differences in the treatment pairs. However, the results indicated that the pre-labelling technique was estimating Ndfr as well as the direct approach apart from the problems of well matched cross-labelling pairs.

In the Krumbach soil/soybean treatment, values of Ndfr estimated using the direct and pre-labelling approaches were not significantly different, again showing that the new approach was giving comparable values to the direct approach. Approximately one third of the N in the crop was derived from the residue, but there was not a significant increase in dry matter or N yield associated with the residue addition. This was probably due to simultaneous immobilisation of inorganic N. Even under these conditions, the new approach gave similar values to the direct technique. Thus, this technique allows estimation of nitrogen derived from residues when there is simultaneous immobilisation and mineralisation of N.

Nitrogen recovery

Percentage of N added as residue recovered in the rye-grass crop-percentage N recovery from alfalfa (20.7%) in the Wechsel soil was the highest of the residues tested, most likely due to its low C:N ratio, as has been previously observed (Hadas et al., 1993; Melillo et al., 1982). The amount of N in the rye grass derived from the residue decreased significantly from the first (20.7 mg) to the second (4.3 mg) harvest suggesting rapid initial mineralisation and uptake of the residue, leaving the more recalcitrant organic N fractions in the soil for subsequent slower mineralisation. Hu et al. (1997) showed that more than 30% of the N in cover crop residues was mineralised within 35 days.

The percentage N recovery of the soybean residue was not significantly different in the Krumbach and Seibersdorf soils, 7.7 and 7.8%, respectively, suggesting similar rates of mineralisation and N uptake in both soil types.

The percentage N recovery from the maize was the less than 1% in both Krumbach and Wechsel soil. The % N recoveries from the common bean (5.9%) and soybean residues were significantly lower than from the alfalfa residues, implying that soybean and common bean residues were mineralised at a significantly slower rate than alfalfa residues. These results demonstrate the influence of C:N ratio on residue decomposability in residues with low polyphenol concentrations, in these experiment % N recovery decreased exponentially with increasing C:N ratio (r^2=0.81 data not shown).

The technique is intended to measure the N release from organic residues, for improved crop production. If there is a significant immobilisation of N leading to extreme yield depression, as in the maize residue treatment, then it may not be considered as a suitable residue for N fertilisation. However, as demonstrated in the Krumbach soybean treatment, the new approach to the pre-labelling method can account for immobilisation, thus overcoming some of the errors associated with pool substitution.

The values of Ndfr obtained using the new approach to the isotope dilution technique gave good agreement with the direct values in most treatments and soil types. The cross-labelling approach allowed easy comparison of the two methodologies. The challenge now is to develop a field pre-labelling procedure that will allow indirect estimations of Ndfr for a variety of organic residues.

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