Symposium no. 59 Paper no. 795 Presentation: poster

The influence of quality parameters on plant N uptake from organic residues

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Abstract

At a time when there is increasing concern about the decline in organic matter content in the tropical soils, the use of multipurpose tree prunings applied to the soil may play a dual role as a fertiliser and as a source of organic matter. Experiments were conducted in the FAO/IAEA-Agriculture and Biotechnology Laboratories, Seibersdorf, Austria, using Krumbach sandy loam to test the influence of plant litter quality parameters on plant N uptake from decomposing leaves of economically important trees and green manure speceies. The direct approach of isotope dilution method was used in these experiments. In the first experiment the leaves were labelled with ^{15}N by tree injection, the labelled material was collected and incorporated at a rate of 100 mg N kg^{-1} soil and sown with ryegrass (*Lolium perenne* L.). Ryegrass was harvested at 67 days and nitrogen derived from the residues (Ndfr) was determined by the direct ^{15}N method. In a second experiment ^{15}N labelled residues of *Casuarina*, *Leucaena*, *Albizia*, *Medicago*, *Gliricidia* and *Eucalyptus* were added at a rate of 100 mg N kg^{-1} soil, ryegrass was grown for 87 days and Ndfr was estimated using the direct ^{15}N method. In the third experiment two months old leaves and twigs from 1.5 year old trees which had been continually fertilised with ^{15}N labelled nutrient solution (low concentrations) were used as dry and fresh materials. Ryegrass (*Lolium perenne*) was planted in soils amended with tree litter already labelled with ^{15}N isotope. The litter *from Acacia auriculiformis, Albizia lebbeck, Gliricidia sepium* and *Eucalyptus grandis* were applied at a rate of 100 mg N kg^{-1} soil as fresh and dry residues . Differences between the two treatments in dry matter yield, total N, and N derived from residue (Ndfr) per pot in the ryegrass were insignificant. But there were significant differences when considering the different tree species. Relationships between residue quality characteristics (lignin, acid detergent fibre -ADF, cellulose and total extractable phenol (TEP) concentrations and the nitrogen derived from residue were determined. TEP concentrations were highly correlated with nitrogen in the ryegrass derived from residues. The correlations with other quality characteristics were not as strong. TEP concentration was the best predictor of plant N release in this experiment. It should also be noted that determination of TEP concentration is a straightforward procedure which could be developed as a simple method for field use by extension workers. Results from this experiment suggests that drying procedure had little or no effect on the dynamics of N release and that the relatively small analytical differences in TEP concentration may not necessarily lead to differences in N release in the soil, or that drying at 70°C overnight

does not affect TEP concentrations significantly.under well-watered glasshouse conditions there is little difference in the mineralisation rates of residues whether added dry or fresh. The dry residues used in this experiment were dried at 70°C and not stored for long before use. Results from the Eucalyptus treatments suggested that there was a lag period, but whether this was due to the breakdown of polyphenols or the establishment of more desirable C:N ratio is unclear. The Acacia treatments showed the lowest N release suggesting that other allelopathic compounds may have been released during breakdown as it did not have a particularly high TEP concentration or C:N ratio.

Keywords: polyphenol, soil, ^{15}N, tree residue, mineralisation

Introduction

The use of leguminous plants as a source of nutrients is increasingly important in many parts of the humid tropics where inorganic N fertilisers have not proved to be economically viable, due to poor markets for fertilisers and lack of infrastructure development (Palm and Sanchez, 1990, Mafongoya *et al.*, 1997). At time when there is increasing concern about decline in organic matter content in tropical soils, the use of multipurpose tree pruning applied to the soil may play a dual role as a fertiliser and a source of organic matter. Three modes of action of residues in enhancing soil quality are recognized: i) direct nutrient supply, ii) indirect effect on the soil microclimate and iii) effect on soil structure. Residues of nitrogen-fixing trees are the most attractive as they have high nitrogen content and thus enhance the availability of soil N (Barrios *et al.*, 1997; Nair 1984; Young 1989). Tree prunnings are potentially important as trees can be used in multipurpose systems, also providing essential fuelwood, shade, etc.

The mineralisation of N from organic residues is dependent on chemical and physical properties of residues, the environmental and edaphic conditions during organic matter breakdown (Heal *et al.*, 1997). It has been shown that initial N, lignin and polyphenol concentrations are the main chemical factors which determine N release from residues (Fox *et al.*, 1990; Palm and Sanchez 1991; Constantinides and Fownes, 1994a). Plant materials with low C:N ratios, lignin and polyphenols are termed high quality litters. They are readily decomposable (Palm and Sanchez, 1991) and are often used as fertilisers. However, high quality litters may also be subject to high N losses through leaching or gaseous losses. "Poor quality" litter with high C:N ratios, lignin and polyphenol concentrations may improve crop performance through mulching effects on the soil microclimate (Palm and Sanchez, 1991; Kachaka *et al.*, 1993). Although there is a body of work relating residue quality to N release using non-isotopic methods (Palm and Sanchez 1991; Constantinides and Fownes, 1994a; Handayanto *et al.*, 1994), there are limited number of studies using direct ^{15}N method to measure plant N uptake from organic residues (Fox *et al.*, 1990; Cadisch *et al.*, 1998). One of the advantages of the isotopic method over the non-isotopic methods is that it is yield independent and allows the direct study of the process controlling decomposition.

A more detailed understanding of the factors controlling organic breakdown is required to make predictions of N release and subsequent plant uptake from organic residues. The importance of the quality of organic additions to N dynamics in natural and managed ecosystems has long been recognized (Melilo *et al.*, 1982). There is a general consensus that net N mineralisation occurs if the residue N concentration is

above 20 mg g^{-1} (2%), and that immobilization occurs below that concentration. Physical factors, such as size and freshness of the residue, are also thought to play a role in the rate of residue decay.

The objectives of the present study were: i) to further investigate the effect of the chemical characteristics of different tree leaf residues on the N release and plant N uptake; ii) to assess the influence of drying the residue on N release and uptake and iii) to investigate the time course of N release from different residues.

Materials and Methods

All experiments were conducted in the FAO/IAEA Agricultural and Biotechnology Laboratories, Seibersdorf, Austria using Krumbach sandy loam. Soil properties were pH (soil:water 1: 2.5) 7.9; total N, 1.1 g kg^{-1}; soil organic matter wet oxidation, 20 g kg $soil^{-1}$ (Hood *et al.*, 1999). Day and night temperatures in the green house were 28and 20 °C, respectively. The light regime ranged from 220-860 nm moles $M^{-1}S^{-1}12$-h^{-1} photoperiod, with relative humidity of 60 – 70% (day and night amplitude).

Experiment 1

The tree leaf residues were labeled using a ^{15}N stem injection method, which allows labeling of the plant without physically disturbing the soil. ^{15}N was injected directly into the xylem vessels element. Three year old *Casuarina equisetifolia* and *Acacia auriculiformis* trees were injected with 5 mL of 9.8 atom % ^{15}N excess as 12 mM ammonium sulphate dissolved in a sap solution of 5.0 mM KCl and 0.4 mM malic acid adjusted to pH 5.4 using the method described by Horwath *et al.* (1992).

Six weeks after injection leaf samples were collected in batches, from the trees, dried at 70 °C, ground to 200 µM and analysed for ^{15}N. Only the batches, which were sufficiently enriched with ^{15}N were used in the experiment.

An additional labeled plant residue, *Gliricidia*, was used in the experiment. Seedlings were planted in Krumbach soil and grown in greenhouse. Soil received 5 atoms % ^{15}N excess ammonium sulphate and after one year the leaves were harvested and prepared as above. The chemical composition of the residues is given in Table 1.

Table 1 Chemical composition of residues used in experiment 1.

Treatment	Residue Type	Constituents (mg kg^{-1})					Atom%	C:N
		ADF[a]	Cellulose	Lignin	TEP[b]	N	^{15}N excess	Ratio
2	*Casuarina* Batch 1	372	256	11.7	55	19.1	0.479	25:1
3	*Casuarina* Batch 2	408	102	15.9	72	15.7	0.347	32:1
4	*Casuarina* Batch 3	331	210	11.8	72	16.3	0.488	31:1
7	*Acacia* Batch 1	356	245	10.9	89	15.4	0.546	32:1
8	*Acacia* Batch 2	373	239	11.4	73	20.6	0.331	24:1
9	*Acacia* Batch 3	310	219	9.6	83	17.7	0.467	27:1
10	*Acacia* Batch 4	310	216	9.8	74	22.7	0.466	22:1
11	*Acacia* Batch 5	373	246	13.3	78	17.1	0.154	24:1
12	*Acacia* Batch 6	347	246	10.5	81	25.4	0.277	26:1
13	*Gliricidia*	190	120	5.0	9	31.8	2.644	13:1

[a] ADF, acid detergent fibre
[b] TEP, total extractible phenol

capacity. Fifteen seeds of ryegrass (*L. perenne*) were sown per pot. Fresh and dry residues were treated as separate treatments with 3 replicates. Pots were arranged in a completely randomized design. A single application of ammoinium sulphate solution containing 9.89atom% ^{15}N excess was made on the same day to control pots at a rate of 100 mg N kg^{-1} soil.

Determination of quality characteristics

Acid detergent fibre (ADF)

Plants were analyzed using a simplification of the CTAB/Sulphuric acid method (Rowland and Roberts, 1994). 0.5 g ± 0.0001g of ground plant material (dried at 70°C) was accurately weighed into an ANKOM (ANKOM USA) filter bag of known dry weight. The filter bags were heat-sealed, labeled and refluxed for one hour with 100 ml CTAB/sulphuric acid solution (100 g cetytrimethyl ammonium bromide in 5 litres of 0.5 M sulphuric acid), anti bumping stones and a few drops of anti foam agent, Octan-2-ol. The filter bags were washed under running dematerialized water, and then with boiling water until there was no foam and the pH of the wash water was neutral. The bags were then washed three times in acetone. The bags were dried at 105°C, then cooled in a desiccator and re-weighed. ADF was determined as the percentage weight loss of the initial sample.

Cellulose

The concentration of cellulose was determined following on the ADF determination. The filter bags were soaked in 50 ml 72% H_2SO_4 for 3 h. They were then washed under running demineralized water, then several times with acetone. The bags were dried at 105°C, cooled in a desiccator and re-weighed. The amount of cellulose was determined by difference, and calculated as the percentage of initial sample weight.

Lignin

Following the cellulose determination the filter bags were ashed for 1h at 550°C in porcelain bowls and re-weighed after cooling in a desiccator. The amount of lignin was determined by difference in weight, and calculated as a percentage of initial sample weight.

Polyphenolics

The following method was adapted from King and Heath (1967) and Allen *et al.* (1974). Total soluble extractable polyphenolics were analyzed by the Folin-Ciocalteu method, and which includes hydrolysable tannins and condensed tannins, as well as non-tannin polyphenolics.

Dried ground plant material (0.3 g) was weighed into a beaker, 20 mL of 50% aqueous methanol added and beakers covered with Para film. Samples were then placed in a water bath at 77-88 °C for 1 h and were then filtered and made up to 50mL.

An aliquot (1 mLl) of extractant was then mixed with 20 mL of distilled-de-ionised water, 2.5mL of Folin-Ciocalteu reagent (Cat No 109001, Merck kgaA, Austria) and 10 mL 17% Na_2CO_3 solution, left to stand for 20 min and the absorbency measured at 760 nm. Standard curves were prepared as above from a tannic acid solution and values were reported in tannic acid equivalent (0.050 g tannic acid standard-Cat No 100773 Merck dissolved in 500 ml water).

Total extractible polyphenolics (mg kg^{-1}) = [(concentration x 5) / mass of sample]x 100

Protein binding capacity was measured using the method of Dawara *et al.* (1988). Aliquots of plant methanol extract (100 µl) were used. The ratio of plant material to methanol was 1g of plant material to 20 ml of methanol-water extract.

Nitrogen, carbon and ^{15}N analysis

All harvested plant material and residues were dried at 70°C to constant weight and then ground to 200 µm. The added residues were analysed for total N, C and ^{15}N by an IRMS Optima Micromass system (Micromass UK, Wythenshaw) linked to a Carlo Erba Strumentazione nitrogen-carbon analyzer 1500 combustion unit (Milan, Italy).

Calculations

Percentage Nitrogen derived from residue (%Ndfr), amount of nitrogen derived from residue and %N recovered from the residue was calculated using the following equations (Hauck and Bremner, 1976):

$$\%Ndfr = \left(\frac{atom\%\ ^{15}N\ excess\ of\ plant\ receiving\ labelled\ residues}{atom\%\ ^{15}N\ excess\ of\ labelled\ residues} \right) x100 \quad (1)$$

$$Ndfr\ (mg) = \frac{\%Ndfr}{100} x\ total\ N\ (mg) \quad (2)$$

$$\%\ N\ recovery\ from\ residue = \frac{Ndfr\ (mg)}{N\ added\ as\ residue\ (mg)} x\ 100 \quad (3)$$

Statistics

All results were analysed using one way ANOVA with $p < 0.05$ indicating a significant difference. The packages Microsoft Excel and Jandel Scientific Sigma stat were used.

Results

Experiment 1

The chemical characteristics of the leaves were similar in *Casuarina* and *Acacia* prunings with both exhibiting low residue quality as defined by Palm and Sanchez (1991). *Gliricidia* exhibited high residue quality characteristics with low polyphenols and high N concentrations (Table 1).

There was a significant decrease in ryegrass dry matter and N yield per pot in all residue treatments compared with the no residues control (Table 3). The ^{15}N enrichment of the ryegrass in all residue treatments was greater than in the control indicating that ryegrass took up N from residues (data not shown). Percentage N recovery from residues was less than 5% of the N added in all the treatments (data extracted from table 3; In this experiment Ndfr (mg N pot^{-1}) was equivalent to % N recovery, as 100 mg N as residue was added to 1 kg of soil).

There was a ten-fold difference in the amount of Ndfr ranging from 3.0 mg pot^{-1} in the *Gliricidia* treatment to less than 0.3 mg pot^{-1} (Table 3). Regression analysis of Ndfr against residue quality characteristics indicated that TEP of the residue gave most significant correlation with Ndfr (Table 4). ADF and N concentration values of the residues were also significantly correlated ($p < 0.05$) with Ndfr. There was no significant

Pinus treatments, ryegrass dry matter yield and N yield were significantly ($p< 0.05$) lower than the control (Table 5). Again in all treatments, the ^{15}N enrichment of ryegrass was significantly greater than the no-residue control at natural abundance (data not shown).

Table 5 Average ryegrass shoot dry matter production (DM), total N, percentage nitrogen derived from residue (Ndfr%) and nitrogen from residues (Ndfr).

Treatment	Residue type	DM (g pot^{-1})	Total N (mg pot^{-1})	Ndfr %	Ndfr (mg pot^{-1})
1	*Pinus*	3.05 (0.05)	31.8 (0.6)	0.6 (0.4)	0.18 (0.01)
2	*Casuarina*	3.83 (0.09)	34.7 (0.7)	2.4 (0.08)	0.83 (0.03)
3	*Leucaena*	4.08 (0.07)	56.9 (1.3)	16.5 (1.01)	9.36 (0.54)
4	*Albizia*	4.21 (0.03)	53.0 (1.7)	14.0 (0.72)	7.43 (0.61)
5	*Medicago*	4.83 (0.03)	71.2 (4.6)	27.6 (1.21)	19.7 (1.800
6	*Gliricidia*	4.67 (0.03)	61.6 (1.6)	26.5 (1.81)	16.45 (1.44)
7	*Eucalyptus*	3.64 (0.02	32.1 (0.3)	1.9 (0.14)	0.47 (0.16)
8	No residue added	4.36 (0.09)	40.7 (0.8)		

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

Ndfr ranged from 20 mg N pot^{-1} in Medicago treatment to less than 0.2 mg N pot^{-1} in the *Pinus* treatment. Again TEP concentration gave the strongest correlation with Ndfr ($p< 0.01$, $R^2= 0.85$) Table 4, and with dry matter yield ($p< 0.001$, $R^2 = 0.925$ (Table 4). The residue characteristics N concentration and C:N ratio also showed significant correlation with dry matter and N yield per pot and Ndfr (Table 4). TEP/N concentration of residue showed strongest correlation against added N yield of ryegrass. Percentage N recovered from residue N added ranged from 20% in the Alfalfa treatment to less than 0.2% in the *Pinus* treatment.

Experiment 3

The highest cumulative dry matter yield per pot was in the control (ammonium sulphate) treatment and lowest in the *Eucalyptus* treatment (data not shown). There were no significant differences in the cumulative Ndfr, dry matter yields or N yields between the fresh and dried residue treatments. Only at 72 and at 135 days in the Acacia treatment and at 93 days in *Gliricidia* treatment was there a significant difference in Ndfr between treatments (Figure 2). *Gliricidia* initially showed high levels of N release, which declined over time, while *Albizia* followed a similar pattern of N release and similar quantities of N mineralized. Ndfr in the *Acacia* treatment was low over the 135-day period peaking at 72 days after planting. In the *Eucalyptus* treatment, Ndfr was initially low and the grass showed obvious symptoms of nitrogen stress. However, Ndfr increased to a maximum after 50 days and then declined again. There was significant ($p < 0.05$) correlation between cumulative Ndfr (mg) and polyphenols concentration of the residues ($r^2= 0.527$). Cumulative recovery from the residues ranged from 33% of the N added in the *Albizia* treatment to 20% of the N added in the *Eucalyptus* treatment.

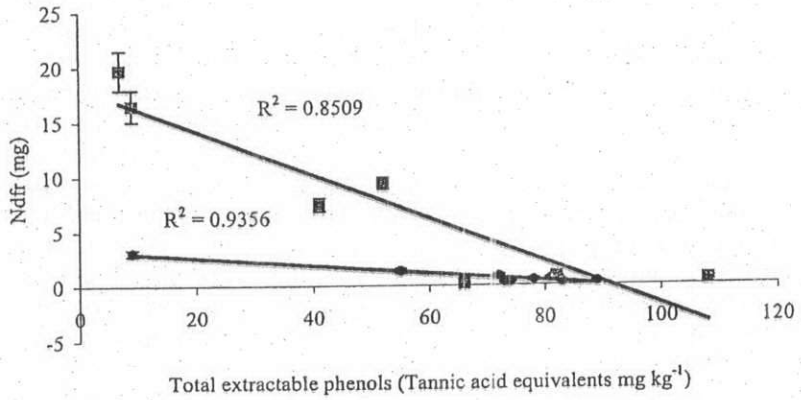

Figure 1 Relationship between nitrogen derived from residues and total extractable phenol concentration for Experiment 1 (•) and Experiment 2 (■).

Discussion.

In experiments 1, 2, and 3, Ndfr was significantly correlated with concentration of polyphenols in he plant residues added. TEP concentration was the best predictor of plant N release in these experiments and exhibited better correlation than previously reported in literature (Fox *et al.*, 1990; Cadisch *et al.*, 1998). These results suggest that polyphenol determination are good primary predictors of N mineralisation in plant material that exceed 3 % tannic acid equivalent threshold, at which the TEP concentration is thought to play a role in N release dynamics (Palm, 1995). It should also be noted that determination of TEP concentration is a straight forward procedure, which could be developed as a simple method for field use by extension workers. Constantinides and Fownes (1994b) showed that measurement of TEP concentration in residue dried at 50°C was considerably less than residue dried at 20°C. However the data from experiment 3 suggests that drying procedure had little or no effect on the dynamics of N release and that relatively small analytical differences in TEP concentration may not necessarily lead to differences in N release in the soil, or that drying at 70°C overnight does not affect TEP concentration significantly. Another explanation is that analytical differences observed by Constantinides and Fownes (1994b) were a result of high temperature polyphenol-protein binding, leading to lower amounts of detectable polyphenols (Markkar *et al.*, 1993). However, these complexes may be weak and not influence N release under normal soil conditions. Unfortunately no polyphenol analysis of fresh residue was carried out in experiment 3.

Protein binding capacity (PBC) of the residue measured in experiments 1 and 2 showed no correlation with Ndfr, DM or N concentration in ryegrass, contrary to results from Cadisch *et al.* (1998) which showed that PBC was a better predictor of N release from residues. The difference in results may be attributed to the drying procedure of the residues or differences in the methods used to measure PBC.

In experiment 1 ^{15}N enrichment of the grass and Ndfr estimates suggested that N was taken up from the decomposing residues, but this resulted in little or no net N

benefit over the zero residue controls. Immobilisation of inorganic N by the residues is the obvious explanation for this, as both the added *Casuarina* and Acacia residues were of low quality. The fact that positive Ndfr results were obtained when there was no net increase in plant N content compared with no N control, suggests that there is an initial immobilization of the inorganic N, followed by N release due to residue breakdown, the extent of N release being dependent on the quality of the residue. If the residue quality is high (High N and low TEP concentration) then there will be net release and both the direct ^{15}N method and total N difference methods will give positive results. If the residue quality is low (low N and high TEP concentration) and there is net immobilization then only the ^{15}N method will demonstrate release and the total N difference method will give negative values. Thus the ^{15}N method is more sensitive to low levels of N mineralisation although it may not be accompanied by net N release. This may explain why better correlations are achieved when quality characteristics are evaluated with ^{15}N method than with the total N difference method. However, Fox *et al.* (1990) proposed that total N difference is the preferred method for measuring N release from residues.

There is some controversy surrounding the ^{15}N technique, because, the addition of residues may lead to "pool substitution" as described by Jenkinson *et al.* (1985) and Hart *et al.* (1986), which cases underestimates of Ndfr (Fox *et al.*, 1990). In experiment 2, the N difference approach indicated a greater N mineralisation than the ^{15}N approach where net N release, as shown by difference, was positive. Another explanation for the discrepancy is that the addition of residues caused a real added nitrogen interaction; i.e additional soil N was released due to increased soil biological activity, as described by Jenkinson *et al.* (1985). This work demonstrates the complexity of apparent verses real added nitrogen interactions still requires further research. It also suggests hat the use of direct ^{15}N approach in conjunction with total nitrogen difference approach leads to greater understanding of the processes taking place.

The *Gliricidia* treatment yielded no N benefit in Experiment 1 whilst yielding a significant N benefit in experiment 2. This may have been due to the longer growing period in experiment 2 leading to further breakdown and N release from residue.

Experiment 3 data clearly indicated that there was no difference between fresh and dry residue treatments. There has been some speculation that drying breaks down polyphenols and changes the structure of residue. However the results from this experiment suggested that under well-watered glasshouse conditions there is little difference in mineralisation rates of residues whether added dried or fresh. It must be stressed that the residue was dried at 70 °C and was not stored for long period before use. The difference in the N release patterns of the residues suggests that the plant quality characteristics played a significant role. Although weak correlation of Ndfr and quality characteristics was observed, this may have been due to the small number of treatments. Results from the *Eucalyptus* treatment suggests that there was a lag period, but whether this was due to the break down of polyphenols or to the establishment of more desirable C:N ratio is unclear. The *Acacia* treatment showed the lowest N release suggesting that other alleopathic compounds may have been released during breakdown as it did not have a particularly high TEP concentration or C:N ratio (Table 6).

Table 6 Initial chemical composition of residues experiment 3.

Treatment	Residue type	N(mg kg^{-1})	Atom% ^{15}N excess	C(mg kg^{-1})	C : N
	Gliricidia	32.0	2.64	401.0	13:1
	Acacia	31.9	3.01	448.0	14:1
	Albizia	34.5	3.82	410.0	12:1
	Eucalyptus	19.3	3.83	431.0	22:1

The results from these experiments clearly demonstrates that total extractible phenols play a significant role in predicting N release from tree residues and adding fresh or dry residue to soil had little or no effect on the N release from residues.

Acknowledgements

We thank Chris Rigney, Gudni Hardarson and Phil Chalk for constructive comments on the manuscript; Leo Mayr, Martina Aigner, Stefan Borovits, Gerhard Eckhardt, Christine Ficker and Nobert Jagoditsch for their invaluable help with the experiments.

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