CHAPTER 3

DEVELOPMENT OF AN *IN VITRO* PERFUSION METHOD TO ESTIMATE RESIDUE BREAKDOWN RATES AND ASSOCIATED NUTRIENT RELEASE

3.1 Introduction

Organic matter is a major determinant of both physical and chemical fertility of soil and is important for the maintenance of an active soil biota (Allison, 1973). Through the breakdown of organic matter by micro-organisms, plant nutrients are supplied directly to plants and to other micro-organisms, and substances are produced which help bind soil particles together to give a stable structure. In addition, an active biota has direct effects on soil aeration and aggregation (Theng *et al.*, 1989).

The processes of organic matter decay are largely controlled by soil micro-organisms and are therefore influenced by temperature, moisture, pH and soil aeration (Jenkinson, 1981). Another factor that affects the breakdown rate is the type of organic matter. Plant material that is low in lignin and other polyphenols, and high in nitrogen and soluble carbohydrates, generally decomposes relatively quickly (Tian *et al.*, 1995b). Thus the rate of initial breakdown varies between immature and mature tissue as well as between species.

Considering the factors which affect organic matter breakdown, it is not surprising that the organic matter content of soils declines rapidly after forests and grasslands are cleared for agricultural production, and that there is increasing interest in using plant residues and other organic materials for improving soil productivity in agricultural systems. To develop the effective use and management of residues, a detailed understanding of decomposition rate and nutrient release is needed, and this requires techniques that can be used to monitor changes in decomposition and nutrient release of residues. Such a technique is reported here.

Plant residue decomposition rates in natural and agricultural systems have most often been determined using litter bags (Wieder and Lang, 1982). These provide an integrated measure incorporating the effects of leaf chemical composition, soil biota and climatic effects. Attempts to design farming systems in which the plant residue breakdown rate can be manipulated to maintain a more continuous supply of carbon for microbial activity and nutrients for both microbes and plants, would be greatly assisted by a standardised, but simple, laboratory procedure for measuring plant residue breakdown rate. This would allow screening of plant residues and ranking of potential materials.

The objectives of the study was to compare results of organic matter breakdown and associated nutrient releases estimated by the *in vitro* apparatus of Nyamai (1992) with a low cost alternative developed in this study at University of New England (UNE), Armidale, Australia.

3.2 Materials and Methods

3.2.1 The UNE (University of New England) Perfusion Apparatus and Its Components.

The UNE perfusion apparatus developed is shown in 3rd. The main components of the apparatus are a sample compartment, a perfusion solution bag, a reservoir for collecting and mixing the perfusion solution before recirculation, a solution sampling tap, a CO_2 trap and a CO_2-free air supply to carry CO_2 evolved during decomposition to the CO_2 trap. Each part of the apparatus is shown in 3rd and 3rd and details described below.

a) **$CaCl_2$ solution bag (part A)** : A 500 mL viaflex container with female luer adaptor, manufactured by Baxter Healthcare Pty. Ltd. was used as the bag. Similar solution sets can be obtained from suppliers of medical equipment.

b) **Solution administration set (part B)** : A medical solution administration set with luer slip adaptor, manufactured by Baxter Healthcare Pty. Ltd. was used to connect between the $CaCl_2$ bag and the sample compartment. This allows $CaCl_2$ from the bag to flow through the plant material in the sample compartment. The in-built flow controller is used to adjust the solution flow rate. Plastic tube (0.4 cm diameter) cut to the required length is used as air inlet tube (part J), air outlet tube (part I), and $CaCl_2$ recirculation tube (part H).

c) **Sample compartment section** : This consists of two lids (part C1 and C2), a stainless steel spring (C3), nylon mesh (0.4mm) (part C4 and C5), sample compartment (C6) and outer part of sample compartment (C7). Three holes (0.4 cm diameter) were drilled in lid C1 and two holes in lid C2, and the two lids glued together (ensuring that two holes in C2 were aligned with two of the holes in C1). Air inlet tube (J) and solution administration set (B) were glued into the aligned holes of lids C1 and C2. The air outlet tube (I) was glued into the third hole of lid C1.

The sample compartment (C6) is made from a 100 mL screw cap polycarbonate sample jar (70x45 mm size). Several holes, 0.25 cm diameter, were drilled in the bottom of this compartment to allow $CaCl_2$ solution to pass through. The plant material to be studied was placed between two layers of nylon mesh in the sample compartment with the spring (C3) between the upper nylon mesh and the lid. The compartment was screwed firmly into lid C2.

The outer part of sample compartment (C7) was made from 250 mL screw cap polycarbonate sample jar (100x65 mm size). A 1 cm diameter hole was drilled at the bottom of the jar to allow $CaCl_2$ solution to pass through to the lower reservoir. This outer part was then screwed firmly into lid C and fitted tightly into the lower reservoir (D).

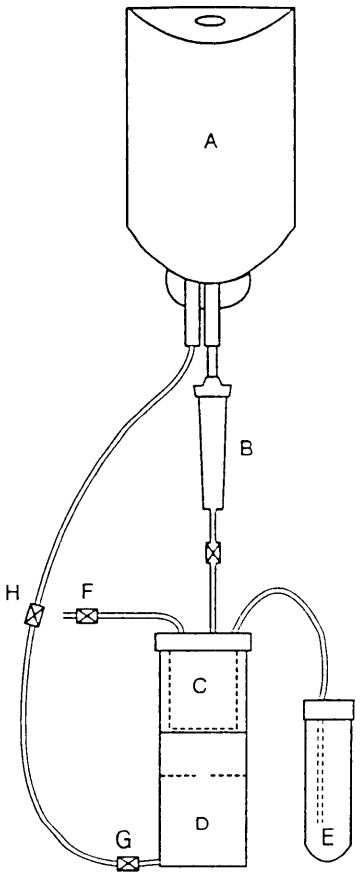

Figure 3.1 : The UNE perfusion apparatus

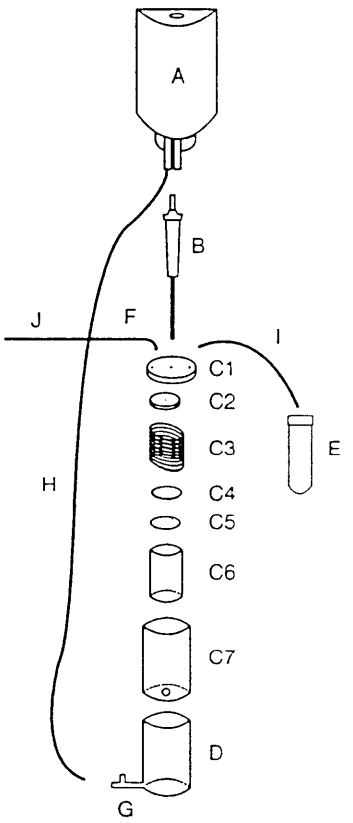

Figure 3.2 : Further details of components of the UNE perfusion apparatus

d) **Reservoir (part D)** : Reservoir was made from the same container as the outer part of sample compartment (C7). A hole was drilled at the bottom of the container and connected with the $CaCl_2$ sampling tap - part G (a 3 way stop-cock luer fitting manufactured by Indoplas Pty. Ltd. was used). The other end of the tap was connected to the solution recirculation tube (H) and this tube connected with the plastic tube from the solution bag. This connected tube allows $CaCl_2$ to recirculate from the reservoir back to the solution bag.

e) **CO_2 trap vial (E)** : A 50 mL centrifuge tube with lid was used. Two holes were drilled in the lid, The air outlet tube (I) was inserted in one hole and the other hole used for ventilation.

f) **Scrubbing agent (F)** : A fish tank air pump was used as an air supply. CO_2 free air was produced by pumping air through sodalime to trap CO_2 before it flowed through system. The CO_2 free air flowed through the main air line and then passed through the air inlet tube of each apparatus. A tap was fitted to each air inlet tube to enable control of air flow into each compartment.

All joints of the assembled apparatus (Figure 3.1) were sealed with silicone to ensure there were no leaks.

3.2.2 Nyamai Perfusion Apparatus and Its Components

The main components of Nyamai perfusion apparatus are sample compartment, solution sampling device, reservoir for collecting and mixing perfusion solution before circulation and adequate air flow rate to provide enough suction pressure for recirculation the $CaCl_2$ solution and a CO_2 trap, all constructed out of glass with plastic tube connectors (3rd). The solutions is circulated by a pneumatic lift system, provided by air pressure, which requires very even pressure in tube 6 and careful positioning of the U-portion of tube 5.

1a : Sample column
1b : Suction device
2 : Solution sampling unit
3 : Reservoir
4 : PVC tube for solution recirculation
5 : Glass tube for solution recirculation
6 : Scrubbed air tube (CO_2-free air)
7 : CO_2 absorption solution
8 : Glass tube containing sodalime

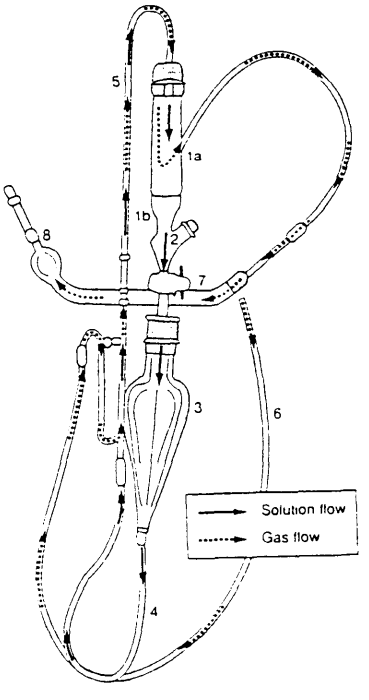

Figure 3.3 : Nyamai *in vitro* perfusion system (Nyamai, 1992)

3.2.3 Experimental Design and Treatments

The UNE and Nyamai perfusion apparatus were compared in a study of the breakdown rate and nutrient release of barrel medic (*Medicago truncatula*) hay. Treatments were arranged as a Randomised Complete Block Design (RCBD). The UNE apparatus was replicated 3 times while the Nyamai apparatus was not replicated due to a shortage of apparatus. A control treatment, where no residue was present in the sample compartment was also included to measure CO_2 in the air introduced into the system. The nutrient composition of the medic hay used is presented in Table 3.1.

The perfusion experiment was carried out for 42 days in a controlled temperature laboratory (approximately 25 °C) at the Department of Agronomy and Soil Science, University of New England, Armidale, N.S.W., Australia.

Statistical analysis was undertaken by calculating the standard deviation of the mean of the 3 replicates of the UNE system and comparing the Nyamai data with this value. The nutrient release data presented in this experiment were calculated from the difference between nutrient content in the initial and in the remaining residues.

Table 3.1 : Nutrient composition of medic hay

Nutrient	Concentration
Nitrogen	2.77 %
Phosphorus	0.21 %
Potassium	1.50 %
Sulfur	0.23 %
Sodium	0.37 %
Magnesium	0.29 %
Calcium	1.66 %
Zinc	9.45 $\mu g\ g^{-1}$
Iron	107.66 $\mu g\ g^{-1}$

3.2.4 Preparation of Plant Residue

The medic hay residue was cut into approximately 2 - 3 cm pieces to achieve an even distribution and oven-dried at 80 °C for approximately 24 hours until the constant weight was achieved. The residue was stored in a desiccator prior to weighing.

3.2.5 Management of the UNE Perfusion Apparatus

a) Management of Residue

A 2.1 g sample of medic hay was weighed and put into the sample compartment of each apparatus between nylon mesh discs to prevent the loss of residue from the compartment. Pressure was maintained on the plant sample in the sample compartment by a stainless steel spring to maintain even movement of $CaCl_2$ through the sample.

b) Management of Perfusion Solution

The perfusion solution bag was filled with 200 mL of 0.005 M $CaCl_2$ solution, a concentration similar to the ionic strength of soil solution. The 200 mL of $CaCl_2$ solution flowed through the sample container each day, by gravity, at a rate of approximately 1 drop per 10 seconds. The solution was collected in the reservoir before being returned manually back to the perfusion solution bag to begin the next cycle. This $CaCl_2$ solution was recirculated once per day and was achieved by placing the $CaCl_2$ bag below the level of the reservoir and opening and closing the appropriate taps. At the completion of the experiment the used $CaCl_2$ solution bag and solution administration set were disposed of due to the difficulty of cleaning and to avoid contamination, however, with careful cleaning the apparatus can be reused.

c) Management of CO_2 -Free Air

CO_2-free air was pumped through each apparatus to enable measurement of the amounts of CO_2 produced by microbial respiration. The rate of flow was standardised in each apparatus by counting the air bubbles in the CO_2 traps, the rate of air flow was approximately 1 bubble per second. The apparatus was checked daily for leaks, reduction of pressure or blockage. A fine granule self-indicating sodalime (0.7 - 1.4 mm size) was used to trap CO_2 in the CO_2-free air supply system and was changed occasionally when its efficiency of CO_2 trapping was low. This was determined by an increase in CO_2 trapped in the blank control treatment.

d) Management of CO_2 Trap

During the first 14 days, vials containing about 35 mL of 0.5 M KOH were used to trap evolved CO_2 and these sample vials were collected and replaced daily. After this period, the rate of CO_2 evolution decreased and the amount and concentration of KOH were reduced to 30 mL of 0.25 M and the vials were collected and replaced every 2 - 3 days. These sample vials were capped tightly and subsequently titrated for CO_2 measurement or, if needed, stored in 2 °C cool room prior to the titration.

3.2.6 Management of the Nyamai Perfusion Apparatus

A 2.1 g sample of medic hay was weighed and placed into the sample column of the apparatus and retained in the sleeve by nylon mesh. The reservoir was filled with 200 mL of 0.005M $CaCl_2$. The $CaCl_2$ solution in reservoir was carried by air pressure to flow through plant material in the sample compartment continuously with the flow rate of 5 mL hr^{-1}. The $CaCl_2$ was collected and mixed in the reservoir prior to subsampling for analysis (Figure 3.3). The CO_2-free air supply and CO_2 trap were managed as for the UNE perfusion apparatus. At the end of the experiment the Nyamai perfusion apparatus was disassembled, washed, acid-soaked for few days, rinsed with deionised water, oven-dried and kept for the next experiment.

3.2.7 Residue Remaining, CO_2 Measurement and Data Analysis

a) Residue Remaining

After 6 weeks of perfusion, the remaining residues were removed from each apparatus, oven-dried in 80 °C until a constant weight was achieved. The residue was then ground to pass 1 mm sieve and stored in a plastic jar for subsequent analyses.

b) CO_2 Evolution

The trapped CO_2 was measured by the method described by White (1979). Fifteen mL of 10% w/v of $BaCl_2$ was added to the KOH sample in a conical flask to precipitate the $CO_3^{=}$ as solid $BaCO_3$ and four drops of phenolphthalein indicator added. The remaining OH^- was then back titrated with 0.5 M HCl until the pink suspension turned white. The volume of HCl used was recorded as T1. One mL of methyl orange indicator was then added to the suspension and titration continued with 0.5 M HCl until the yellow solution just turned pink, at this stage the $BaCO_3$ had dissolved and the final volume of HCl used was recorded as T2. Then amount of CO_2 trapped was calculated by using the formula below;

$$CO_2 \text{ evolved (mg day}^{-1}) = \frac{(T2 - T1) \times 22 \times M}{t}$$

where;

T1 = Amount of HCl used to neutralise KOH

T2 = T1 + Amount of HCl used to dissolve precipitated $BaCO_3$

M = Molarity of HCl.

22 = 22 mg CO_2/1 mL 1M HCl

t = Time in days

The CO_2 in the control treatment, which had no residue, was subtracted from the calculated value for CO_2 release.

c) Estimation of $CaCl_2$ Losses

Prior to starting the perfusion experiment, the amount of $CaCl_2$ in each apparatus was weighed and recorded. The whole apparatus, including $CaCl_2$ and residue, was then weighed to obtain the total weight of each apparatus. Each week, after 10 mL $CaCl_2$ was removed for analysis and replacement with 10 mL of 0.005 M $CaCl_2$, the whole apparatus was weighed and recorded. Thus, the $CaCl_2$ remaining each week was obtained by subtract the weight of apparatus in the current week with the weight from previous weeks, with a correction of amount of CO_2 lost from the residue. By performing this calculation for each successive week, the volume of $CaCl_2$ remaining was obtained. These volumes were graphed against time and subsequently, the linear regression equation was obtained as

$$Y = -0.2604X + 199.91$$

$$R^2 = 0.996$$

where; Y= Volume (mL) of $CaCl_2$ remaining in the apparatus

X=Time in days

The volume of $CaCl_2$ lost was estimated from this equation and used in calculating the total amount of nutrient released.

d) Plant Residue Analyses

i) Determination of Macronutrients and Micronutrients

The residue remaining at the end of the experiment, as well as samples of the initial plant residues, were digested using the Sealed Chamber Digest method described by Anderson and Henderson (1986). A sub sample (about 0.2 g) of ground plant residue was weighed into an acid washed polycarbonate vial. Two mL of a 7:3 (v/v) mixture of $HClO_4$ (70%) and H_2O_2 (30%) was added to each vial and capped lightly. After pre-digestion for a minimum of 2 hours at room temperature, 1 mL of H_2O_2 was added, the vial tightly sealed and placed into an oven at 80 °C for 30 minutes.

After removal from the oven, the sample vial was allowed to cool slightly and a further 1 mL of H_2O_2 was added, capped tightly and returned to the oven at 80 °C for a further 1 hour. If further digestion of the sample was required, the sample was digested with 1 mL aliquots of H_2O_2 at 30 minutes intervals in an 80 °C oven. After the digestion was completed, the sample vial was allowed to cool, made to approximately 25 mL volume with distilled/deionised water and mixed thoroughly. The sample extract was then filtered through a 0.45 µm glass fibre filter (Whatman filter paper No 42) and the concentration of cations, phosphorus and sulfur were determined by Inductively Coupled Plasma Atomic Emission Spectroscopy (ICP-AES).

The difference between initial and final nutrient concentration and dry weight was deemed to be nutrient release.

ii) Determination of Carbon and Nitrogen Content

The N and C concentration in the plant residues was determined using the Automatic Nitrogen and Carbon Analyser/Mass Spectrometer (ANCA-MS) System. This consists of a Carlo-Erba NA 1500 dynamic flash catalytic combustion automatic carbon and nitrogen analyser as a continuous flow preparation unit, coupled to an Europa Tracermass Isotope Ratio Mass Spectrometer.

3.3 Results

3.3.1 Carbon Release

There was no significant difference in the rate of carbon released or total amount of carbon released from the residues between the UNE and Nyamai perfusion methods. After 42 days, an average of 49.8% of carbon had been released from the medic hay (Table 3.2).

Table 3.2 : Carbon released from medic (*M. truncatula*) hay as determined by the UNE and Nyamai perfusion systems

Parameters	Perfusion Systems	
	UNE apparatus	Nyamai apparatus
Carbon (mg)	450.3 ± 37.2	459.7
Carbon (% of added)	49.7 ± 3.9	49.8

Note : n = 3 for UNE apparatus, n = 1 for Nyamai apparatus

3.3.2 Pattern of Carbon Release

The pattern of daily CO_2 release from the medic hay studied by the two perfusion systems was very similar. Daily CO_2 release of both systems reached a maximum value at day 3 and gradually decreased after this time (Figure 3.4). After 42 days, 49.8% and 49.7% of added residue carbon had been released from Nyamai and UNE systems respectively and there was no significant difference between the two systems (Figure 3.5).

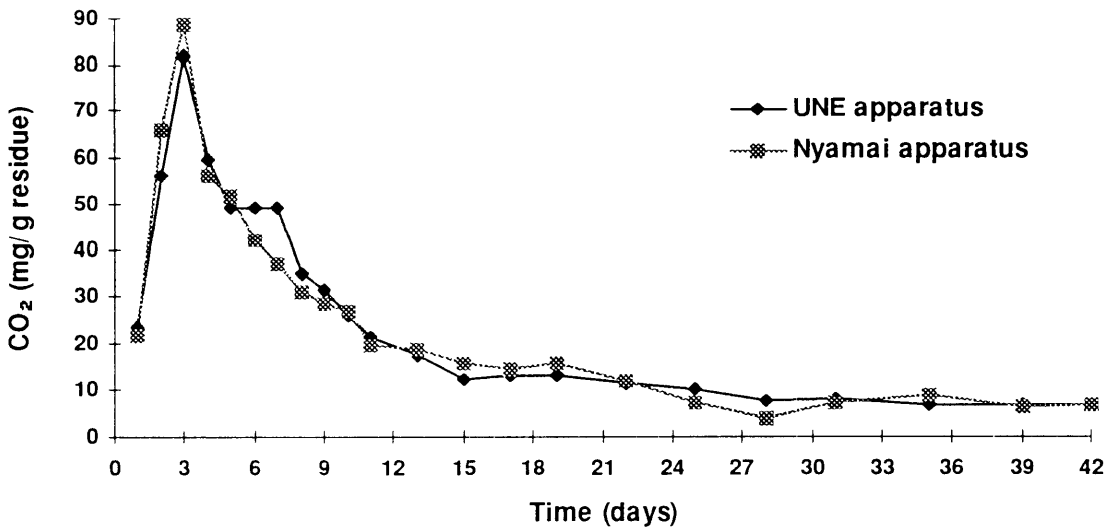

Figure 3.4 : Daily CO_2 release from medic hay (*M. truncatula*) as determined by the UNE and Nyamai apparatus

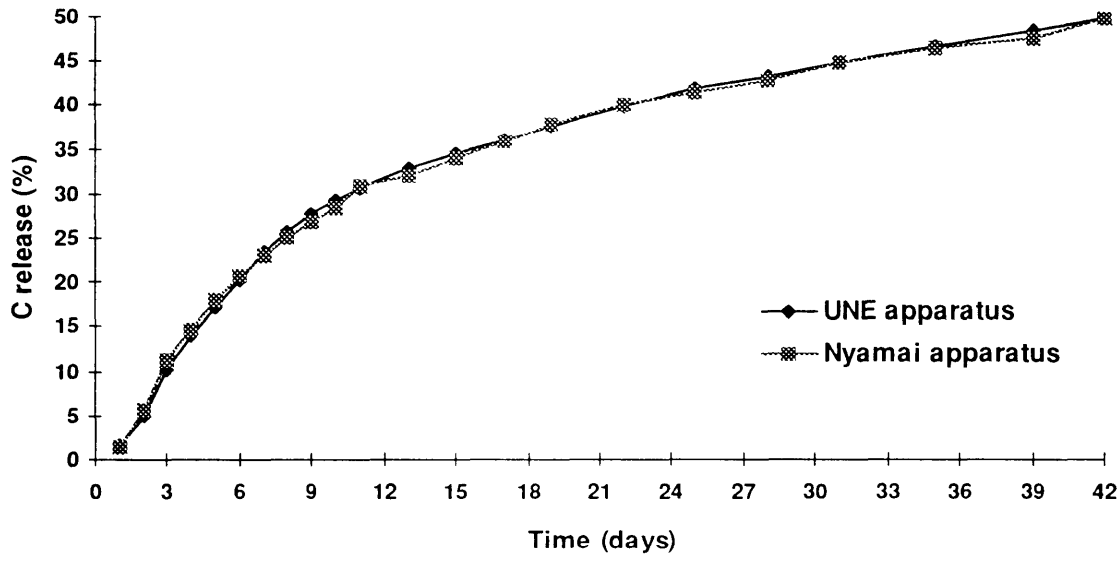

Figure 3.5 : Cumulative carbon release from medic hay (*M. truncatula*) as determined by the UNE and Nyamai apparatus.

3.3.3 Nutrient Release

There was no significant difference between the two perfusion systems in the released (Table 3.3) proportion of N, P, S, K, Mg and Mn. There was, however, a significant difference in the proportion of sodium released between the two (Table 3.3).

Table 3.3 : Nutrient released as % of that added in medic (*M. truncatula*) hay as determined by the UNE and Nyamai perfusion apparatus

Nutrient	Release of % added	
	UNE apparatus	Nyamai apparatus
Nitrogen (%)	44.6 ± 2.8	46.5
Phosphorus (%)	38.3 ± 10.6	37.5
Sulfur (%)	52.6 ± 4.3	51.3
Potassium (%)	89.2 ± 1.6	90.8
Magnesium (%)	82.3 ± 3.8	81.9
Sodium (%)	98.1 ± 0.6	96.7
Manganese (%)	31.8 ± 10.2	24.1

Note : n = 3 for UNE apparatus, n = 1 for Nyamai apparatus

There were marked differences between nutrients in the proportion of nutrient released from the residues ranging from 24.1% for Mn up to 90.8 for K and 96.7% for Na in the Nyamai apparatus (Table 3.3).

3.4. Discussion

The close agreement between the two methods means that either can be used to study breakdown rates. The primary advantage of the UNE system are that it is easier and cheaper to construct and easier to operate compared to the Nyamai apparatus. With the UNE apparatus it is easier to assemble and manage sufficient units to conduct statistically valid comparisons between large numbers of residues or between treatments, such as where the same residues are treated with additions of different chemicals or inoculated with different soil suspensions.

CHAPTER 4

DECOMPOSITION OF PLANT RESIDUES WITH DIFFERENT QUALITY AND THE IMPACT ON GROWTH AND YIELD OF TWO SUCCESSIVE WHEAT CROPS

4.1 Introduction

The cropping systems employed in many agricultural areas in the tropics are characterised by low, and often variable yields, large responses to fertiliser application, but very low efficiency of utilisation of applied fertiliser. When fertiliser is applied much can be lost through leaching and erosion. Fertilisers are often not applied, or not applied at the appropriate time or in adequate amounts because of other constraints such as soil moisture, or because of inadequate economic returns.

There is a great deal of evidence that the decline in crop yield with continued production in many agricultural areas is correlated with declines in organic matter levels (Norman, 1984 as cited by Boonchee and Anecksamphant, 1993). As organic matter declines yields can only be maintained by significant inputs of fertilisers (Sanchez *et al.*, 1987) until, in many soils, the efficiency of utilisation of fertilisers is so low that the benefit : cost ratio is less than 1. To improve the stability and productivity of these systems, the organic matter content of the soil needs to be increased. Increasing the soil organic matter provides a reservoir of plant nutrients and also improves the soil nutrient and moisture holding capacity. Significant benefits from increased residue return can also arise from reduced soil erosion and suppression of weed growth. However, the timing of crop residue addition to the system, and the residue decomposition rate, has implications for the subsequent crop and the efficiency of nutrient recovery.

In the past, organic matter management to increase soil organic matter has generally been approached via the use of green manuring and crop residue return. Application of green manure often results in increases in crop yield, however, there was no evidence of long-term improvement of soil organic matter and soil fertility (Herrera *et al.*, 1989). Green manuring often produces short term pulses of nutrients into the farming systems, resulting from extremely rapid release of C and nutrients due to the rapid breakdown of the added material. Conversely, lower quality crop residue return (e.g cereal straw) releases nutrients too slowly, and in insufficient quantities to meet microbial and crop requirements. As an alternative, the application of high quality residues which breakdown relatively

slowly due to chemical and/or physical restrictions, could result in significant inputs of carbon, provide other nutrients for the longer periods and reduce soil nutrient losses. This is especially apparent in tropical systems where high temperatures increase decomposition rates and high rainfall may cause significant leaching losses. Small inputs of fertiliser may, however, be required at planting to overcome short term nutrient deficits. Considering this, the use of slower degrading plant residues as an organic matter source may be a promising alternative to maximise the efficiency of the C and nutrient cycles in agricultural systems.

To examine this hypothesis, a pot experiment was conducted to investigate crop response to application of plant residues with a wide range in quality. The shrub legumes *Flemingia macrophylla* and *Albizia chinensis* were used as sources of slowly mineralising organic matter, while chickpea (*Cicer arietinum*) straw and medic (*Medicago truncatula*) hay represented rapidly mineralising organic matter. The straw residue of the test crop, wheat (*Triticum aestivum*), was also evaluated.

The UNE perfusion apparatus described in Chapter 3 was used to evaluate the breakdown rate and nutrient release of these plant residues and to compare these results with those found in the pot experiment. The objectives of the study were;

1. To study the pattern of decomposition and nutrient release of different residues in the *in vitro* UNE perfusion apparatus.
2. To study the growth response and nutrient uptake in a pot trial of two successive wheat crops supplied with different quality plant residues.
3. To compare the *in vitro* and plant growth measurements as techniques for evaluating different plant residues as soil amendments.

4.2 Materials and Methods

Two separate experiments were conducted to study the breakdown and nutrient release from plant residues with different quality. In the first experiment the UNE *in vitro* perfusion apparatus (as described in Chapter 3) was used to study the breakdown and nutrient release of plant residues. The second experiment was a pot experiment undertaken to study the impact of decomposition of the same residues used in the perfusion experiment in soil on the yield and nutrient content of two successive wheat crops.

4.2.1 Experiment 1. Release of Carbon and Nutrients from Crop Residues and Green Manures Crop Determined in the UNE Perfusion Apparatus.

a) Experimental Treatments and Design

The UNE perfusion system was used to study the breakdown and nutrient release of 5 plant residues with different quality, namely;

1. Wheat (*Triticum aestivum*) straw
2. Leaf litter from 2 tree legumes representing slow breakdown residues;

- *Flemingia macrophylla* leaf
- *Albizia chinensis* leaf

3. Plant residues of 2 legume crops representing rapid breakdown residues;
   - Barrel medic (*Medicago truncatula*) hay
   - Chickpea (*Cicer arietinum*) straw

Hereafter referred to as wheat straw, *Flemingia* leaf, *Albizia* leaf, medic hay and chickpea straw, respectively.

Treatments were arranged as a Randomised Complete Block Design (RCBD) and were replicated 3 times. A control treatment, where no residue was put into the sample compartment, was also included. The chemical composition of the plant residues are presented in Table 4.1.

Table 4.1 : Chemical composition of crop residues used in the perfusion experiment

Residue	N (%)	C:N ratio	P (%)	S (%)	K (%)	%ADF[A]	% Lignin	%Tannin[B]
Wheat	0.298	138.3	0.030	0.042	0.291	58.52	8.48	<1
Flemingia	2.642	16.4	0.230	0.305	1.193	47.95	20.24	2.18
Albizia	4.398	10.4	0.322	0.266	1.318	35.08	16.83	5.74
Medic hay	3.178	13.5	0.225	0.224	1.448	38.04	6.95	<1
Chickpea	3.594	11.1	0.267	0.231	1.624	20.76	3.82	<1

[A] Fiber content determined by the Acid-Detergent method (Van Soest, 1963)
[B] Tannins are expressed as vanillic acid equivalents (Broadhurst and Jones, 1978).

b) Management of Perfusion Experiment and Statistical Analysis

The residues were cut into small pieces (1 - 2 cm) and oven-dried at 80 °C for approximately 24 hours or until constant weight was achieved. A 3.5 g sample of the appropriate residue was put into the sample compartment of the apparatus. The management of the apparatus, measurements and data collection were the same as those described in Chapter 3.

The perfusion experiment was carried out for 84 days in the controlled temperature laboratory (approximately 25 ºC) at the Department of Agronomy and Soil Science, University of New England, Armidale, N.S.W., Australia.

Data were analysed by analysis of variance using the NEVA computer program (Burr, 1982) and the differences between treatment means deemed to exist when they were significant at the 5% level ($P < 0.05$) of probability. The nutrient release data presented in this experiment was calculated from the difference between nutrient content in the initial material and in the residue remaining after 84 days.

4.2.2 Experiment 2. A Glasshouse Study of the Effect of Residues on Crop Growth and Apparent Nutrient Recovery

a) Experimental Design and treatments

The treatments consisted of a combination of the same five residues used in the perfusion experiment treatment and two residue application rates (3 and 15 t ha^{-1}). The application rate

equivalent to 3 t ha^{-1} most closely approximates the levels of residue applied in practical farming systems. The rate of 15 t ha^{-1} was included to evaluate the effect of an extreme rate of addition of residue on wheat growth and nutrient dynamics within the system. A non-residue control treatment was also included. The factorial combination of treatments were arranged in a Randomised Complete Block Design with 3 replicates. In summary, the treatments were;

[(Five plant residues x Two rates of residue application) + Non residue control] x 3 replicates

- Wheat straw
- *Flemingia* leaf
- *Albizia* leaf
- Medic hay
- Chickpea straw

- 3 t DM. ha^{-1}
- 15 t DM. ha^{-1}

b) Soil and Pot Preparation

The soil used in this experiment was a previously cropped Red Earth (Alfisols) soil from the University of New England's Douglas MacMaster Research Station at Warialda, in northern New South Wales, Australia. The collected soil was air-dried, large pieces of residue were removed, and the remaining soil passed through a 3 mm sieve before being used in the experiment.

A 2 kg of air-dried soil sample was weighed into plastic bags and placed into plastic pots (15 cm in internal diameter and 13 cm deep). No drainage was provided from the pot. The chemical properties of the soil are presented in Table 4.2.

Table 4.2 : Analysis of the MacMaster Research Station cropped soil used in the experiments

Soil Characteristics	Values
Clay Content (%)	13.2
pH (1:5 Water)	5.6
pH (1:5 $CaCl_2$)	4.7
Organic Carbon (%)	0.7
Nitrate Nitrogen (mg kg^{-1})	6.0
Sulfate Sulfur MCP (mg kg^{-1})	1.0
Phosphorus Collwell (mg kg^{-1})	13.0
Potassium (meq $100g^{-1}$)	0.5
Calcium (meq $100g^{-1}$)	3.0
Magnesium (meq $100g^{-1}$)	1.2
CEC (meq $100g^{-1}$)	4.9
Aluminium (meq $100g^{-1}$)	0.11
Sodium (meq $100g^{-1}$)	<0.05
Chloride (mg kg^{-1})	11.0
Electrical Conductivity (dS m^{-1})	0.04
Copper (mg kg^{-1})	0.6
Zinc (mg kg^{-1})	0.2
Manganese (mg kg^{-1})	69.0
Iron (mg kg^{-1})	60.0
Boron (mg kg^{-1})	0.5

Source: Soil mineral analysis by Incitec Ltd, Port Kembla.

c) Plant Residue Preparation and Application

In the plus residue treatments, plant residues were cut into approximately 2 - 3 cm pieces to achieve an even distribution throughout the soil and oven-dried at 80 °C for 48 hours before application. The appropriate residue was applied to the appropriate pot at a rate equivalent to 3 t ha^{-1} and 15 t ha^{-1} dry matter on the basis of pot surface area. Some data on chemical composition of the residues are presented in Table 4.3.

Table 4.3 : Nutrient concentration and amount rate of N addition of plant residues added to the soil

Residue	P (%)	S (%)	K (%)	N (%)	C:N ratio	Residue N (kg ha^{-1})	
						3 t ha^{-1}	15 t ha^{-1}
Wheat	0.026	0.051	0.277	0.14	281.3	4.3	21.3
Flemingia	0.152	0.145	0.726	2.35	19.7	70.4	352.2
Albizia	0.143	0.244	1.093	2.69	18.1	80.7	403.7
Medic	0.165	0.193	1.807	2.23	19.7	66.8	344.1
Chickpea	0.176	0.138	1.591	1.54	26.6	46.1	230.7

Because of low availability of *Flemingia*, a different batch of *Flemingia* leaf was used for the perfusion experiment and the pot experiment, although both litters were grown under similar glasshouse conditions. The same batches of chickpea straw, medic hay and *Albizia* leaf were used, but the main stems or main branches had been screened out prior to use in the perfusion apparatus in order to achieve an easy management, while these were included in this pot trial.

d) Nutrient Application

A complete nutrient solution (excluding nitrogen) was applied such that all treatments received the same amount of each nutrient. The sources of nutrients applied and the rate at which they were applied, in mg pot^{-1} for each compound, and the equivalent kg ha^{-1} for each nutrient, on a surface area basis, are given in Table 4.4. Each of the nutrient sources was dissolved separately in distilled water and was applied and mixed into the soil in the plastic bag prior to the application of plant residues.

Table 4.4 : Nutrient sources and rate of application

Nutrient	Sources	Application rate	
		mg source pot^{-1}	kg Nutrient ha^{-1}
P	K_2HPO_4	88.35	8.88
K	K_2HPO_4		11.22
S	$MgSO_4.7H_2O$	53.01	3.90
Mg	$MgCl_2.6H_2O$ + $MgSO_4.7H_2O$	39.89 + 53.01	5.66 + 2.93
Na	$Na_2MoO_4.2H_2O$	0.044	0.00
Mo	$Na_2MoO_4.2H_2O$		0.01
Mn	$MnCl_2.4H_2O$	1.765	0.28
Zn	$ZnCl_2$	3.543	0.96
Cu	$CuCl_2.2H_2O$	0.353	0.07
B	H_3BO_3	0.883	0.09

In this study, two successive wheat crops were grown to investigate the initial and residual effects of plant residue application. No nitrogen was added to either crop but basal nutrients (Table 4.4) were applied for the first crop prior to wheat planting.

e) Crop Management

After placing the soil into the pot, six wheat (*Triticum aestivum*) seeds were planted into each pot to a depth of approximately 1 cm. A week after emergence, the seedlings were thinned to leave 3 healthy seedlings per pot. The pots were placed in a glasshouse at the Department of Agronomy and Soil Science, University of New England the temperature of which was maintained between 20 and 35 °C throughout the experiment. All pots were watered each day to field capacity by weight and re-randomised every two weeks to avoid bias that may arise as a result of location.

The wheat plants were harvested 10 weeks after planting. At harvest, the wheat plants were cut at soil surface level and the tops were separated into grain and stem + leaf. The plant parameters recorded were dry weights of stem + leaf, grain and plant height. The plant parts were oven-dried at 80 °C until a constant dry weight was achieved, ground to pass a 1 mm sieve and stored in plastic jars. After harvest the soil bag was removed from the pot and the soil was then thoroughly mixed inside the bag, and visible wheat roots removed. Approximately 100 g of soil was taken from each bag, air-dried, ground to pass through a 1 mm sieve and stored in a plastic jar for analyses. The remaining soil was mixed thoroughly and returned to the pot in the plastic bag. The soils were re-wetted and 6 wheat seeds were planted. These pots were then kept in the same glasshouse with the same conditions as for the first crop. The experimental procedures, measurements and data collection were the same as in the first crop, except that no basal nutrients were applied in this second crop. The second wheat crop was harvested 12 weeks after planting.

f) Chemical Analyses for Plant and Soil Samples

A sub-sample of each wheat plant component and plant residues were taken (0.2 g), digested in a sealed container using the method of Anderson and Henderson (1986) as detailed in Chapter 3 and the plant digests were measured for the contents of cations, phosphorus and sulfur by ICP-AES. A sub-sample of wheat plants, plant residues as well as soil samples were also taken for total nitrogen and carbon content determinations using the using the Automatic Nitrogen and Carbon Analyser/Mass Spectrometer (ANCA-MS) System.

Fibre and lignin contents in plant residues were determined by the Acid-Detergent method (Van Soest, 1963). Condensed tannins were determined using the Acidified Vanillin method (Broadhurst and Jones, 1978).

g) Data and Statistical Analysis

Data were analysed by analysis of variance using the NEVA computer program (Burr, 1982) and the differences between treatments means deemed to exist when they were significant at the 5% level by Duncan's Multiple Range Test ($P < 0.05$ of probability). An example of a NEVA output file is shown in Appendix 4.1.

Productivity data and the percentage of apparent recovery of each nutrient in the wheat tops were determined relative to the control treatment using the formula below;

$$\text{Apparent Recovery (\%)} = \frac{(Nt - Nc) \times 100}{Nr}$$

Where; Nt = Nutrient content in the tops of treated wheat plants

Nc = Nutrient content in the tops of wheat plant in the control treatment

Nr = Nutrient content of added residue

In order to examine the correlation between the perfusion and pot trial results, the carbon and nutrient release data from the perfusion experiment were multiplied by the application rates used in the pot trial so that mg of C and N released from the residue could be used in the correlation. Chickpea straw was omitted from the correlation due to its apparent allelopathic effect on the wheat crop when this residue was applied, particularly at the rate of 15 t ha^{-1}. The correlation was performed on only the first crop due to the similar duration of decomposition; 12 weeks and 10 weeks for the perfusion and pot trials, respectively.

4.3 Results

4.3.1 Perfusion Experiment

a) Breakdown Rate of Different Plant Residues.

The initial decomposition rates of the plant residues, excluding wheat straw, are generally high and increased to a maximum 3 – 8 days after the commencement of perfusion, before decreasing rapidly (Figure 4.1 and Figure 4.2). Wheat straw decomposition rate was relatively constant from days 1 – 7, with an average release of 5.5 mg CO_2 g^{-1} day^{-1}, before decreasing slowly. The pattern of breakdown differed between residues. Chickpea straw and medic hay had the highest initial rate of CO_2 evolution, reached a maximum rate of 69.8 and 53.3 mg CO_2 g^{-1} day^{-1} respectively on day 3, and then decreased rapidly. After 84 days, the chickpea straw and medic hay had decomposed the most, with 58.9 and 55.8 % of C released, respectively (Table 4.5).

Table 4.5 : Carbon released after 84 days from the decomposition of plant residues

Residue	CO_2 released (mg)	C released (%)
Wheat straw	595.0 d[A]	11.9 d
Flemingia leaf	1234.5 c	23.3 c
Albizia leaf	2179.4 b	39.7 b
Medic hay	2732.2 a	55.4 a
Chickpea straw	2788.3 a	58.9 a

[A] numbers in the column followed by the same letter are not significantly different

CO_2 evolution from *Albizia* leaf reached a maximum of 32.3 mg CO_2 g^{-1} day^{-1} on day 8 which was later than for the other residues, except wheat straw. After 84 days, 39.7% of total carbon had been released from the *Albizia* leaf. *Flemingia* leaf and wheat straw had slower decomposition rates

than the other residues, with only 23.3% and 11.9% of carbon being released from the *Flemingia* leaf and wheat straw, respectively.

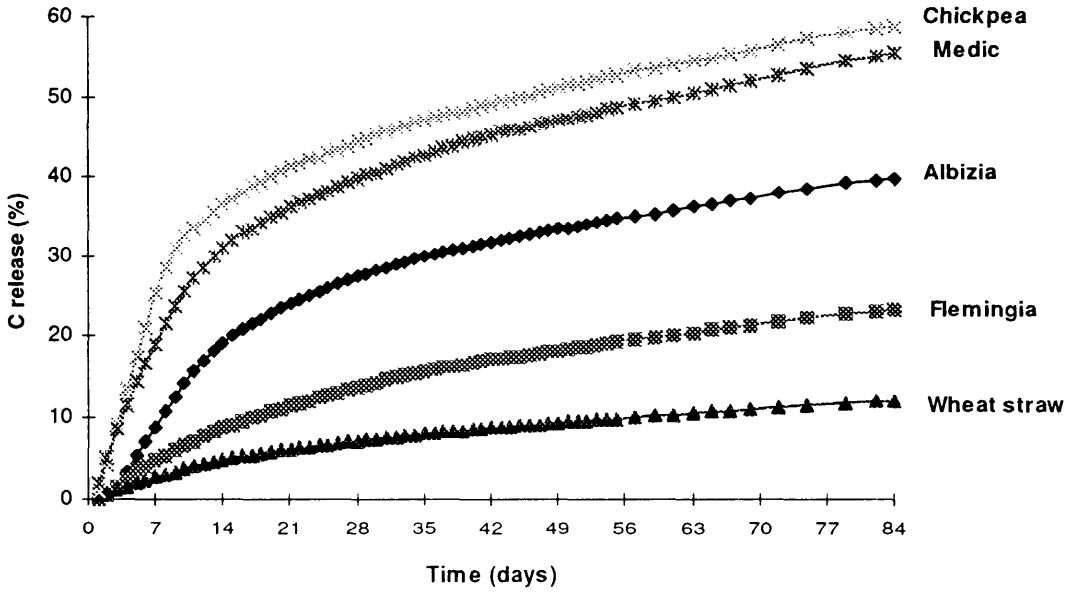

Figure 4.1 : Cumulative carbon released during decomposition of plant residues

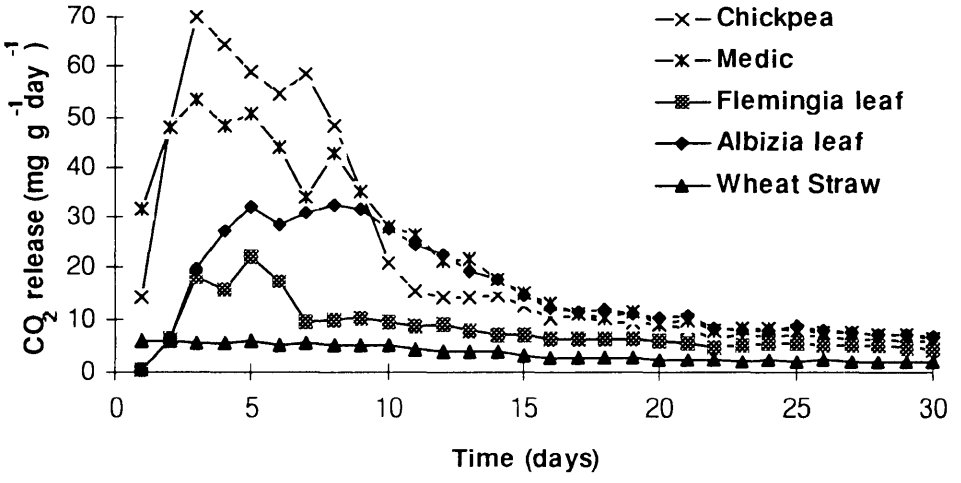

Figure 4.2 : Decomposition rate of plant residues

b) Nutrient Release

The percentage of nutrient released from each residue is presented in Table 4.6. The release varied depending on the type of plant residue and nutrient. The variation between residues was greatest for N and S. On average, percent release was least for S, N and P, and greatest for K. After 84 days, a total of 66.3% and 65.4% of N was released from chickpea straw and medic hay respectively and this was significantly higher compared to the release from wheat straw, *Albizia* leaf and *Flemingia* leaf respectively (Table 4.6).

Approximately 50% of P was released with the highest release from chickpea straw (57%) followed by *Albizia* leaf, medic hay, *Flemingia* leaf and wheat straw (42%). A total of 95.2% of K was released from chickpea straw and this was significantly higher than from the other residues. Potassium release decreased in the order, medic hay, *Albizia* leaf, wheat straw and *Flemingia* leaf respectively although the latter still released more than 85% of the total K (Table 4.6).

Table 4.6 : Percentage of nutrient release from the different plant residues studied by the perfusion technique

Residues	N (%)	P (%)	K (%)	S (%)	Mg (%)	Na (%)
Wheat straw	47.5 b	41.5 b	86.5 cd	39.3 b	85.1 a	66.1 c
Flemingia leaf	14.5 c	50.4 ab	85.4 d	44.3 b	75.0 d	78.4 b
Albizia leaf	37.6 b	55.5 a	87.3 c	17.2 c	69.0 e	84.7 b
Medic hay	65.4 a	53.5 a	91.3 b	58.5 a	79.7 c	94.0 a
Chickpea straw	66.3 a	56.8 a	95.2 a	56.8 a	82.7 b	81.3 b

Numbers in the column followed by the same letter are not significantly different according to DMRT

The release pattern of Mg and S were similar, with higher release from chickpea straw, medic hay and wheat straw, while *Albizia* leaf released the lowest amount of Mg and S. Medic hay released 94% of Na after 84 days and this was significantly higher compared to *Albizia* leaf, chickpea straw, *Flemingia* leaf and wheat straw (Table 4.6).

4.3.2 Glasshouse Experiment

a) First Wheat Crop

i) Wheat Dry Matter Yield

Compared to the non-residue control treatment, wheat growth was retarded when wheat straw was added and this suppression of growth was greater when the application rate was increased to 15 t ha^{-1} (Figure 4.3). In all other residue treatments, application of 3 t ha^{-1} resulted in greater growth of wheat, relative to the control. When the rate of application was increased to 15 t ha^{-1}, only the medic hay and *Albizia* leaf treatments showed an increase in response with wheat dry matter yield more than double that of the control treatment. In contrast, increasing the application rate of *Flemingia* leaf and chickpea straw led to a decrease in wheat yield.

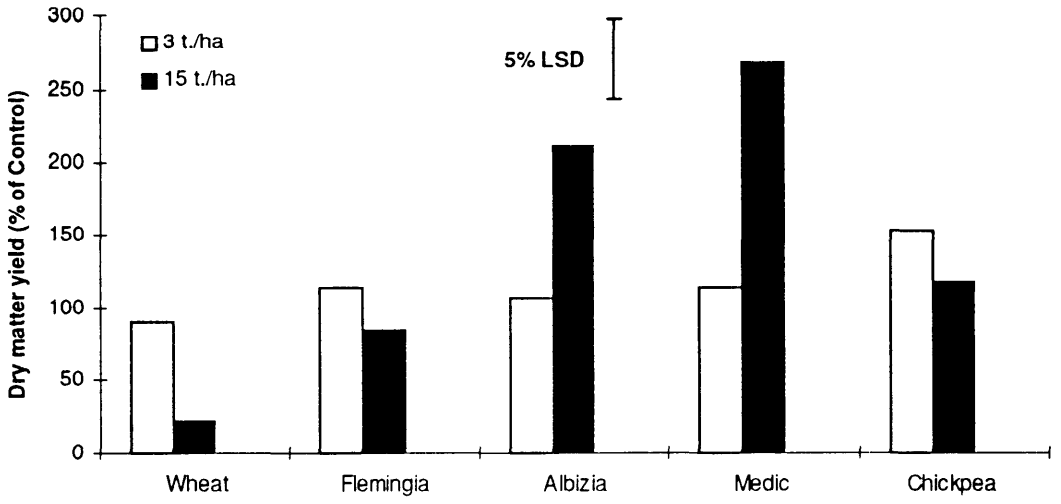

Figure 4.3 : Dry matter yield of the first wheat crop with different rates and types of residues relative to the Control treatment (control = 100)

ii) Plant Nutrient Tissue Content

The apparent recovery of nutrients is shown in Table 4.7. The most obvious feature of this table is the net immobilisation of most nutrients when wheat straw was applied at either rate, with greater immobilisation at the higher rate. The extent of the immobilisation also varied between nutrients. The apparent nutrient recovery from chickpea straw at the 3 t ha^{-1} level of application exceeded that at the higher rate and all other residue treatments. Increasing the application rate of chickpea straw had a detrimental effect on yield, which is reflected in the apparent nutrient recovery of the wheat plants. A similar effect occurred with the *Flemingia* leaf treatment. In contrast, the application of medic hay and *Albizia* leaf at the higher rate resulted in increased apparent nutrient recovery compared to the lower rate.

Table 4.7 : Apparent recovery (%) of nutrients in the first crop[A]

Nutrient	Rate (t ha^{-1})	Wheat	*Flemingia*	*Albizia*	Medic hay	Chickpea
N	3	0.7 b[B]	20.0 b	5.0 b	9.7 b	77.7 a
	15	-7.3 b	2.7 b	7.0 b	12.0 b	3.1 b
P	3	-6.3 ab	23.7 a	16.3 a	23.0 a	36.6 a
	15	-55.9 b	2.7 a	30.7 a	32.7 a	11.0 a
S	3	-74.6 c	2.0 a	-10.7 ab	5.3 a	26.4 a
	15	-44.1 bc	-3.3 ab	12.3 a	14.0 a	7.4 a
K	3	-63.3 b	14.0 a	6.7 a	12.7 a	31.4 a
	15	-58.1 b	-5.3 a	31.3 a	21.7 a	5.7 a
Ca	3	-3.7 a	3.0 a	-2.3 a	0 a	1.4 a
	15	-6.1 a	0 a	2.7 a	1.3 a	0.9 a
Mg	3	-11.7 c	3.7 a	-0.7 abc	4.3 a	11.5 b
	15	-10.0 bc	-0.3 abc	6.7 a	8.3 a	1.8 ab
Na	3	-3.0 b	8.3 a	-1.0 b	0 ab	0.6 ab
	15	-0.9 b	-1.0 b	0.7 ab	0.7 ab	-1.0 b
Fe	3	6.3 b	17.0 a	0.0 c	0.3 c	2.3 bc
	15	-1.2 c	1.3 bc	4.0 bc	3.7 bc	0.8 c
Zn	3	21.3 ab	9.7 ab	21.3 ab	0.7 ab	28.2 a
	15	-17.3 b	2.0 ab	25.3 ab	0.7 ab	5.8 ab
B	3	15.7 a	17.7 a	-6.7 c	1.3 bc	2.7 bc
	15	-5.8 c	1.0 bc	7.7 ab	5.7 abc	0.9 bc

[A] Apparent recovery (%) = [(Nutrients in treated wheat - Nutrients in control wheat) x 100] / (Nutrients added in residues).
[B] For each nutrient, numbers followed by the same letter are not significantly different at 5% level by DMRT.

iii) Soil Nitrogen and Carbon

Application of plant residue resulted in an increase in soil N compared to the control treatment (Table 4.8). Moreover, increasing the application rate of the residues resulted in an increase in soil total N. Among the five residues, application of *Albizia* leaf, medic hay and *Flemingia* leaf increased soil N more than chickpea straw and wheat straw application.

Similar results to soil N were found for soil C (Table 4.8). Soil C was increased when plant residues were added and the increase was greater when the application rate was increased. Among the five residues, soil C increased more when *Flemingia* leaf, *Albizia* leaf and wheat straw were applied, particularly at the higher application rate.

Table 4.8 : Soil C and N receiving different levels of the different residues relative to the Control treatment (control = 100[A])

Parameter	Application rate (t ha^{-1})	Wheat	*Flemingia*	*Albizia*	Medic hay	Chickpea
			Recovery relative to control (%)			
Soil N	3	108.9 e	136.6 cde	162.1 abc	117.0 de	124.1 de
	15	130.4 de	167.4 abc	185.7 a	169.6 ab	147.8 bcd
Soil C	3	144.4 cd	144.0 cd	153.4 bcd	105.5 e	134.2 d
	15	177.0 ab	192.3 a	172.4 abc	155.6 bcd	138.1 d

Numbers in the rows followed by the same letter within a nutrient are not significantly different at 5% level by DMRT.
[A] Soil N in the Control = 0.075% and soil C in the Control = 0.815%

b) Second Wheat Crop

i) Dry Matter and Grain Yields

Compared to the control, growth of the second wheat crop was reduced, as for the first crop, when wheat straw was applied, with a greater reduction when applied at 15 t ha^{-1} (Table 4.9 and Figure 4.4). In all other residue treatments, increasing the residue application rate resulted in an improvement in both dry matter and grain yield production, especially where *Albizia* leaf and *Flemingia* leaf had been applied. Application of *Albizia* and *Flemingia* leaf resulted in increases in wheat yields of more than 4 and 5 times, respectively, compared to the control (Figure 4.4).

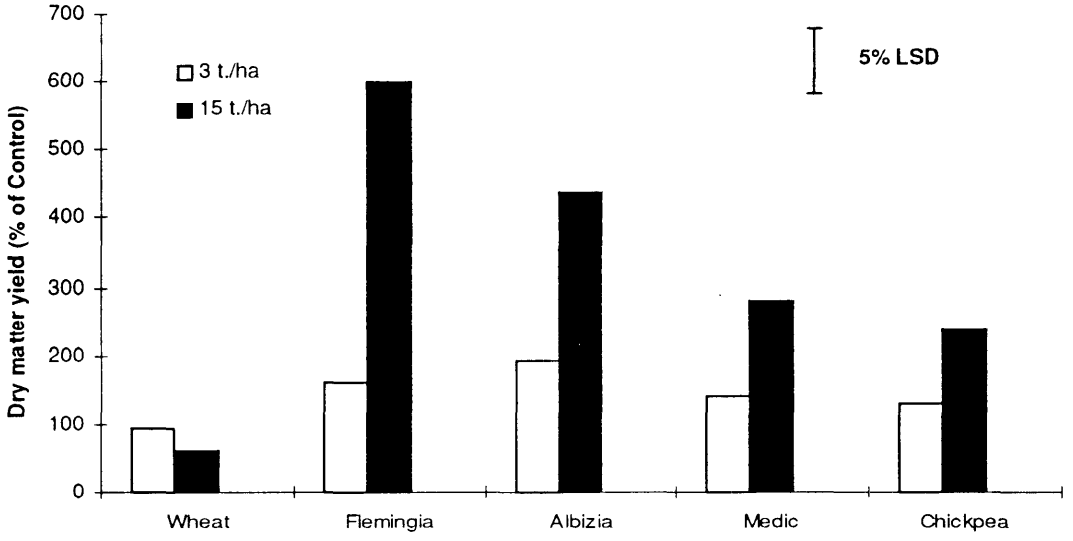

Figure 4.4 : Total dry matter yield of the second wheat crop receiving different levels of different residues, relative to the control treatment (Control = 100).

Table 4.9 : Dry matter and grain yield of the second wheat crop with different levels of different residues, relative to the control treatment (Control = 100[A]).

	Straw yield		Grain yield	
	Residue application rate (t ha^{-1})			
Residues	3 t ha^{-1}	15 t ha^{-1}	3 t ha^{-1}	15 t ha^{-1}
Wheat straw	93.8 ef	49.0 f	96.8 de	78.0 e
Flemingia leaf	167.5 de	575.8 a	151.7 cde	636.3 a
Albizia leaf	201.6 cde	427.6 b	183.8 cde	448.2 b
Medic hay	150.9 ef	294.7 c	124.8 de	264.8 c
Chickpea straw	136.9 ef	262.7 cd	119.5 de	210.9 cd

Numbers within columns followed by the same letter are not significantly different at 5% level by DMRT.
[A] Dry matter yield of the Control = 0.82 g pot^{-1} and grain yield = 0.61 g pot^{-1}

ii) Plant Nutrient Tissue Content

The apparent recovery of nutrients from residues is shown in Table 4.10. Only those nutrients which were significantly different across all treatments are included in the table.

Increasing the application rate of wheat straw prior to crop 1 to 15 t ha^{-1} resulted in lower apparent recovery of most nutrients in the second crop. Application at 3 t ha^{-1} however led to an increase in the recovery of N, P, S and K, but not Mg and Ca. In the *Flemingia* leaf treatment, the higher initial application rate led to higher apparent recovery of all nutrients by the second wheat crop. This is in contrast to chickpea straw, medic hay and *Albizia* leaf litter where increasing the application rate resulted in reduction in the apparent nutrient recovery in the second crop (Table 4.10).

Table 4.10 : Apparent recovery (%) of nutrients in the second wheat crop

Nutrient	Rate	Wheat	*Flemingia*	*Albizia*	Medic hay	Chickpea
N	3	17.3 a	9.3 ab	9.7 ab	5.3 ab	4.3 ab
	15	-4.0 b	13.0 ab	10.0 ab	4.3 ab	4.0 ab
P	3	23.0 cd	39.3 bcd	72.3 a	25.3 cd	16.3 cd
	15	-13.7 e	64.7 ab	43.7 bc	13.3 de	14.0 cde
S	3	62.7 a	56.3 ab	43.3 abc	25.7 cd	32.3 bcd
	15	-13.3 e	60.3 a	27.7 cd	12.0 d	17.0 d
K	3	16.0 cd	45.7 b	46.3 b	14.3 d	12.0 d
	15	-11.7 e	80.3 a	35.0 bc	9.3 d	10.0 d
Ca	3	-1.0 cd	2.3 b	5.3 a	1.3 bc	1.0 bc
	15	-3.3 d	2.3 b	3.0 ab	1.3 bc	1.0 bc
Mg	3	-3.7 d	8.7 abc	14.7 a	6.3 c	7.3 abc
	15	-5.3 d	14.3 ab	11.7 abc	6.7 bc	5.3 c
Mn	3	-20.3 c	7.0 bc	13.0 b	43.7 a	-1.7 bc
	15	-13.0 b	16.7 ab	2.0 bc	6.3 bc	0 bc
Fe	3	-49.7 bc	-52.7 c	-3.0 abc	2.7 ab	-15.0 abc
	15	-19.0 abc	-2.7 abc	-9.3 abc	12.0 a	-0.7 abc
Zn	3	-2.7 c	10.7 bc	48.7 a	0 c	10.0 bc
	15	-6.3 c	29.0 ab	32.7 ab	1.0 c	9.0 bc

For each nutrient, numbers followed by the same letter are not significantly different at 5% level by DMRT.

iii) Soil Nitrogen and Carbon

Additions of plant residues prior to the first crop resulted in increased soil N, after the second crop compared to the control treatment. At the 3 t ha^{-1} rate the relative soil N of all residue application treatments was comparable. Application of *Albizia* leaf and *Flemingia* leaf at the higher rate dramatically improved soil N compared to the other three residues (Table 4.11).

Compared to the control treatment, soil C after the second crop was greater when plant residues were applied with the increase greater at the higher application rate. Among the five residues at the low application rate, soil C was comparable. At the high rate, however, application of *Flemingia* leaf, *Albizia* leaf and wheat straw resulted in a greater increase in soil C compared to medic hay and chickpea straw (Table 4.11).

Table 4.11 : Soil C and N receiving different levels of different residues relative to the control treatment (Control = 100[A])

Parameter	Application rate (t ha^{-1})	Wheat	*Flemingia*	*Albizia*	Medic hay	Chickpea
			Soil N and C relative to control (%)			
Soil N	3	106.7 c	113.0 c	112.6 c	114.4 c	109.0 c
	15	115.3 c	155.6 ab	161.0 a	127.4 bc	122.4 c
Soil C	3	123.1 a-d	142.6 ab	102.1 d	110.8 bcd	108.9 bcd
	15	138.7 abc	156.3 a	141.3 abc	119.8 bcd	108.2 cd

For each nutrient, numbers in the rows followed by the same letter are not significantly different at 5% level by DMRT.
[A] Soil N in the Control = 0.074% and soil C in the Control = 0.906%

4.3.3 Correlation between Perfusion and Pot trial Results

Relationships between the results from the perfusion study and the pot trial have been examined in two correlations, namely, the relationship between carbon release during perfusion and wheat dry matter yield (Figure 4.5) and N release during perfusion and wheat dry matter yield (Figure 4.6).

C and N release measured during perfusion and wheat dry matter yield were well correlated, with coefficients of determination (r^2) of 0.72 and 0.86 respectively (Figures 4.5 and 4.6).

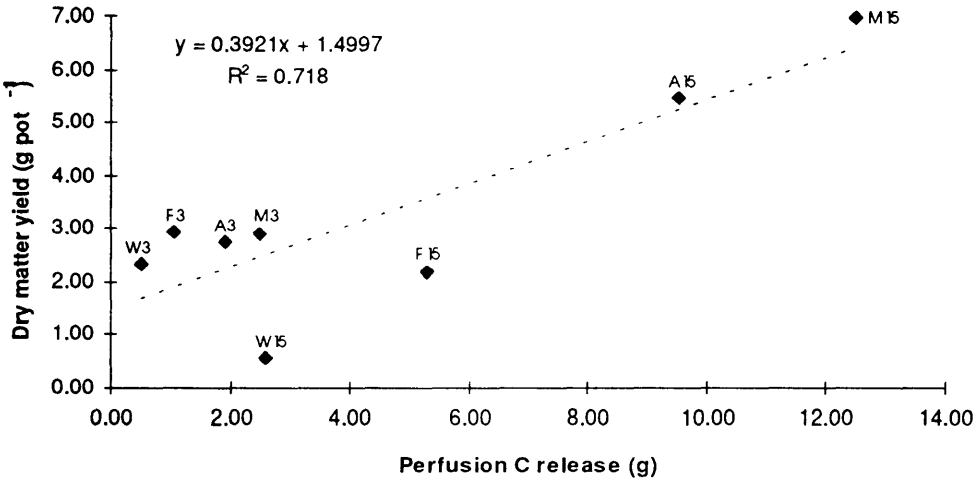

Figure 4.5 : Relationship between carbon released during perfusion and wheat dry matter yield A = *Albizia* leaf, F = *Flemingia* leaf, M = Medic hay, W = Wheat straw, 3 = 3 t ha^{-1}, 15 = 15 t ha^{-1}.

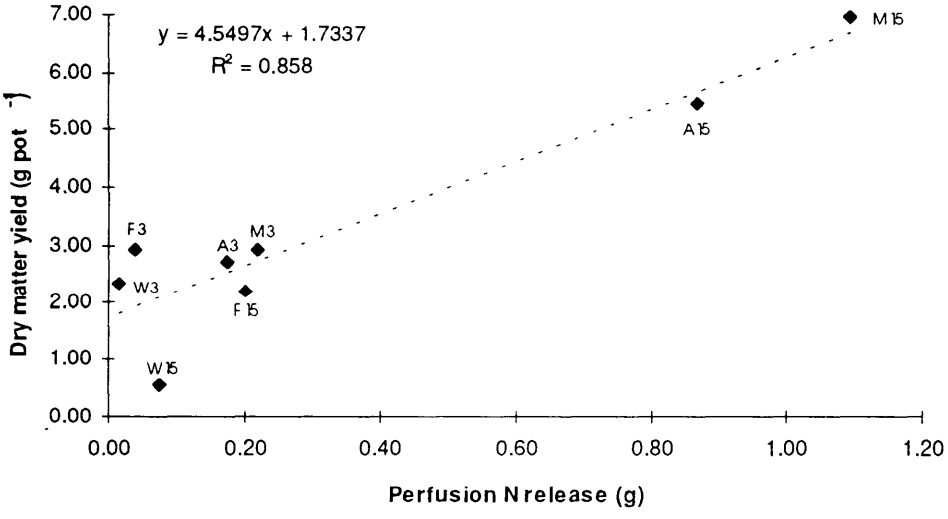

Figure 4.6 : Relationship between N release during perfusion and wheat dry matter; A = *Albizia* leaf, F = *Flemingia* leaf, M = Medic hay, W = Wheat straw, 3 = 3 t ha^{-1}, 15 = 15 t ha^{-1}.

4.4 Discussion

4.4.1 Perfusion Technique

a) Decomposition Rates

The patterns and total amounts of C released from the residues varied considerably, depending on the type of organic material used. In general, the amount of C released from residue as CO_2 was rapid during the initial stages of perfusion, followed by a rapid decline and subsequently slower, relatively linear release rate. High breakdown rate during early stages have been attributed to the decay of readily decomposable fractions of plant carbon (Knapp *et al.*, 1983; Reinertsen *et al.*, 1984; Christensen, 1985). Over longer periods, decay rates decrease due to an increase in the

proportion of relatively stable fractions of residue carbon (Jenkinson, 1977; Sauerbeck and Gonzales, 1977; Ladd *et al.* 1985). Data from the perfusion study (Figure 4.2) confirms this pattern of the decomposition process.

Chickpea straw and medic hay had the highest overall decomposition rates. After 84 days, 58.9% and 55.4% of carbon, respectively, had been released from these two residues, followed by *Albizia* leaf, *Flemingia* leaf and wheat straw. The C:N ratio of the residues were 10.4, 11.1, 13.5, 16.4 and 138.3 for *Albizia* leaf, chickpea straw, medic hay, *Flemingia* leaf and wheat straw, respectively. The amount of CO_2 released is only partially related to the C:N ratio. *Albizia* leaf had a slightly narrower C:N ratio than medic hay or chickpea straw, yet the percentage of C released was less than half that of the medic hay. This contrasts with the observations of Mote and Griffis (1980), Christensen (1986), Keya (1975), Reinertsen *et al.*, (1984), and Nyamai (1992), who found a close relationship between C:N ratio and decomposition rate.

The differences in decomposition rates could also be due to differences in the tenacity of the leaf cuticle and/or the presence of bio-inhibitory organic compounds in the residues. Tian *et al.* (1992a), Berendse *et al.* (1987), and Cochran (1991) found that organic compounds such as lignin and polyphenol, as well as silica, had significant effects on decomposition, which explained some of the differences in breakdown rate not explained by the C:N ratio. The high mechanical strength, lignin and polyphenol (condensed tannin) contents of *Albizia* leaf and *Flemingia* leaf would be expected to reduce accessibility to microbes, and thus the breakdown rate. Such an effect has been observed in animal feeds undergoing rumen digestion (Norton, 1982).

Tian *et al.* (1995b) used nitrogen, lignin and polyphenol concentration to calculate a Plant Residue Quality Index (PRQI). Polyphenol analysis was not available in this study so condensed tannin were used as a surrogate in calculating PRQI in the original equation of Tian *et al.* (1995b) as follows: PRQI = [1/(0.423 C:N + 0.439 Lignin + 0.138 Tannin)] x 100.

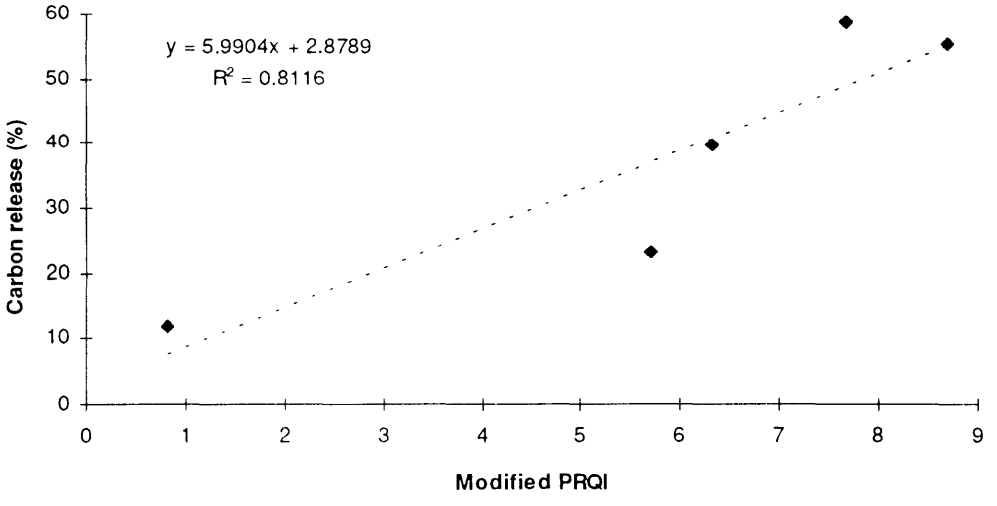

Figure 4.7 : Relationship between C release after 12 weeks in the Perfusion apparatus and modified PRQI.

There was a strong positive relationship (r^2 = 0.81) between measured C release after 12 weeks in the perfusion apparatus and the modified PRQI as show in Figure 4.7.

The results from this work supports the conclusion of many authors such as Tian *et al.* (1992a), Tian *et al.* (1995b), and Cortez *et al.* (1996), who all concluded that the lignin and polyphenol contents in residue must be taken into account, along with C:N ratios, in predicting residue decomposition rates.

b) Nutrient Release

Nitrogen release from plant residues was closely related to the rate of decomposition, except for wheat straw. The release of N also had a close relationship (r^2 = 0.90) with lignin content of the residue. The rapidly decomposing materials, such as chickpea straw (3.82%) and medic hay (6.95%), which contain low amounts of lignin, released the highest amounts of N. In contrast, *Flemingia* leaf and *Albizia* leaf, which contain lignin of 20.24 and 16.83% respectively, released lower amounts of N. Nitrogen release is known to be strongly affected by the chemical composition of leaves such as lignin and polyphenol (Muller *et al.*, 1988; Tian *et al.*, 1992a). Tian *et al* (1992a), Palm and Sanchez (1991), and Vallis and Jones (1973) found that release of N increased with increasing N content and decreased with increasing contents of polyphenols and lignin in leaves.

Whilst only 11.9% of C was released from the wheat straw, 47.5% of the N was released. However, it must be remembered that this is a large proportion of a very small pool of N; only 0.3%. Wheat straw studied in this experiment has a high fibre content (58.52%) but contains a low amount of lignin (8.48%) and condensed tannin (less than 1%). The lower decomposition rate probably represents the high content of fibre, which is difficult to decompose by microbes. The relatively higher release of N is possibly due to the lower content of lignin and polyphenol, resulting in the rapid catabolism of simple soluble-C compounds initially present in the wheat straw, such as simple sugars and amino acids (Lynch, 1979). This agrees with the findings of Hunt (1977) and Herman *et al.* (1977), who showed that the C mineralisation potential of a substrate was directly correlated with its carbohydrate content but inversely proportional to its lignin content. If N was a major limitation to the breakdown of wheat straw, then the release of N would have been low.

The release of S also showed a high correlation with the decomposition pattern, except for *Albizia* leaf. Excluding *Albizia* leaf, the relationship between the percentage of C release and the percentage of S release had an r^2 = 0.98. Stevenson (1986) indicated that S release may be related to the chemical nature of the decomposing fractions. Whilst low nutrient content may be limiting breakdown in some plant residues, high polyphenol and lignin content could be the major factor limiting decomposition and sulfur release from *Albizia* leaf.

There was a good correlation between release of K and pattern of C release (r^2 = 0.75) and the rapid release was observed from all plant materials, including wheat straw. More than 85% of K had been released from the residues, with over 95% of the initial K content being released from chickpea straw. Potassium release appears less affected by the leaf anatomy or chemical characteristics of the residue than N. K is not incorporated into organic compounds and is present in

the cytoplasm and vacuole which allows it to be easily leached from the residues. This agrees with the observations of many authors, such as Thomas and Asakawa, (1993), Kalburtzi *et al* (1990), Anderson *et al.*, (1983), and Palm and Sanchez (1990).

The P release patterns demonstrate the importance of substrate quality on nutrient release. The release of P from residue was related to its initial P content and N:P ratio, with the r^2 of 0.90 and 0.69 respectively. Wheat straw, *Flemingia* leaf, *Albizia* leaf, chickpea straw and medic hay had N:P ratios of 9.9, 11.5, 13.7, 13.5 and 14.1 respectively and 41.5, 50.4, 55.5, 56.8 and 53.5% of P was released. This is in agreement with Palm and Sanchez (1990) who studied the P release from tropical legumes using litter bags and found that nitrogen controlled P dynamics.

Magnesium and Na release did not show a clear relationship with either the initial content in residue (r^2 = 0.45 and 0.60 respectively) or nitrogen release rate. No explanation can be given for the lack of relationship between initial sodium and magnesium content of leaves and sodium and magnesium releases rates.

c) Evaluation of UNE Perfusion Apparatus

The *in vitro* procedure outlined here can be used to rank plant materials in terms of potential breakdown rate and accessibility of nutrients. Recent field studies (Matta-Machado *et al.*, 1994) have shown that decay rate constants for N were best correlated with lignin content of the plant residues. Tian *et al.* (1995b) have developed a plant quality index using C:N ratio and lignin and polyphenol concentration. The procedure described here provides an additional assessment of the "quality" of plant residues which integrates the various controlling factors. Generally, whilst it is agreed that breakdown rate is controlled by a number of controlling factors, including nutrient content and content of lignin and polyphenol, there is considerable debate regarding the best way to measure these organic compounds. While this debate continues, the perfusion technique has considerable attraction as it integrates the controlling factors.

4.4.2 Glasshouse Experiment

a) The First Wheat Crop

i) Wheat yield and nutrient uptake

As is apparent from the results presented, an increase in the rate of plant residue applied does not immediately produce an increase in the crop growth or increase in the mineralisation of nutrients, especially nitrogen. Previous experiments have found that incorporation of plant residues into the soil can result in effects varying from positive (Kumazawa, 1984; Broadbent, 1965; Wonprasaid *et al.*, 1995; Almendras and Serohijos, 1995), to neutral (Armstrong *et al.*, 1996; White, 1984; Conteh, 1994), to negative (Jessop and Stewart, 1983; Cochran *et al.*, 1977). Whether the effects of these additions is positive, neutral or negative depends on the balance between mineralisation and immobilisation (Blair and Boland, 1978). The net amount of nutrient release varied with the ease of decomposition of the organic matter and the amount present. The ease of decomposition of organic matter is related to its C:N ratio, N content (Cadisch *et al.*, 1993), organic

compound content (Zhu *et al.*, 1984) and physical nature of residue (Tian *et al.*, 1992a; Almendras and Serohijos, 1995). Thus, in turn the mineralisation and immobilisation of nutrient in added residue depends on the C:N ratio, organic compound content and the physical nature of the residues.

In this study, application of wheat straw retarded the wheat crop growth at both rates. The decrease in the growth of wheat amended with wheat straw (C:N ratio = 281.3) resulted from nutrient immobilisation which occurred with most nutrients and this effect increased when the application rate was increased. Decomposition of residues with low N contents (high C:N ratio), such as wheat straw, usually results in microbial immobilisation of soil and fertiliser N (Cadisch *et al.*, 1993). This subsequently leads to a reduction in N availability to plants, which results in unacceptable crop performance. A similar result was observed by Parnas (1975) who studied the relationship between N immobilisation and C:N ratio and concluded that N immobilisation occurs if residue has a C:N ratio more than 30 because all the N is utilised by micro-organisms resulted in a lowering of mineral N reserves. The wheat straw used in the experiment had a higher C:N ratio than this critical value. This is also in agreement with results reported by Saini (1989) on the N immobilisation of rice straw.

Application of *Flemingia* leaf at the low rate increased wheat growth compared to the control. Increasing the rate of application of this residue, however, retarded wheat growth which resulted from the low mineralisation of most nutrients, including nitrogen, compared to the low application rate. As can be seen from the results of the perfusion study, *Flemingia* leaf had the lowest decomposition rate and N release of the legume residues. A positive response in wheat yield when *Flemingia* leaf was applied at the low rate suggests that N release from the readily decomposable fractions is adequate for microbes to use as a substrate for growth. Kachaka *et al.* (1993) incubated *Flemingia macrophylla* in soil to study the decomposition and reported that about 74% of total C in *Flemingia* leaf is in the active cellulose and hemicellulose fractions and that this fraction will be decomposed rapidly if there are favourable conditions for decomposition. He also stated that 24% of added N was mineralised from this residue after 112 days of incubation and that this mineralised N enhanced the crop performance. Increasing the application rate from 3 to 15 t ha^{-1} in the present study, most likely resulted in a proportionately lower increase in microbial population which would have resulted in a reduction in the breakdown of carbon pools, such as lignin and polyphenol. As a result N probably became the limiting factor for microbial growth. This agrees with Broadbent and Bartholomew (1948) who reported that the rate of decomposition of oat straw in soil was found to be inversely related to the quantity of straw added. They further commented that the slow rate of decomposition at the high rate of addition cannot be explained on the basis of inadequate supplies of air or of deficiencies of N and P.

Unlike *Flemingia* leaf, increasing the application rate of *Albizia* leaf doubled the productivity of the wheat plants. According to the perfusion experiment, *Albizia* leaf had a 16% higher decomposition rate and 23.1% higher N release than *Flemingia* leaf. In addition, *Albizia* leaf had the highest N content (lowest C:N ratio) which was favourable for microbial attack. This possibly resulted in a high N release to the microbes, especially from the readily decomposable fraction. The high proportion of N release from this fraction was likely adequate for the microbial growth and activity when the increased amount of residue was added. A similar observation was reported by Ladd *et al.*

(1983) who studied the decomposition rate of *Medicago littoralis* and found that the greater amount of plant material added, the greater its decomposition.

Chickpea straw and medic hay application at both rates were found to increase the wheat growth compared to the control. Chickpea straw and medic hay have very favourable C:N ratio of 26.6 and 19.7 respectively in addition to low concentrations of lignin and condensed tannin. This resulted in rapid residue decay rates and subsequent nutrient release that was readily available for crop uptake. This can be observed from the higher mineralisation of most nutrients from chickpea straw compared to the other residues at the low application rate which subsequently led to the high wheat yield. This result supports the observations of Tian *et al.* (1992b, 1993).

Increasing the rate of application of medic hay led to increased crop yield and this was a reflection of the higher mineralisation of all nutrients compared to the low rate. This agrees with Ladd *et al.* (1983). The increased mineralisation and subsequent crop yield probably resulted directly from increased in biological activity. Allison (1973) found that biological activity increased 10 - 50 fold when easily decomposable organic matter was added to the soil. Conversely, Broadbent and Batholomew (1948) reported that decomposition of sudan grass was not proportional to the quantity added and decomposition of oat straw was inversely related to the quantity added. These contrasting observations are probably due to the different residues studied or alternatively the residues studied contained different chemical compounds and nutrient status which led to the differences in breakdown rate. Generally, legumes have a lower C:N ratio and fibre content compared to non-legume crop residues, resulting in higher breakdown rates.

Increasing chickpea straw application rate led to a reduction in wheat growth. This was most likely due to an allelopathic effect which can be apparent when a readily decomposable residue is applied at a high rate. This adverse effect to crop caused by incorporation of plant residue was reported by many researchers (Cannell and Lynch, 1984; Cochran *et al.*, 1977; Putnam, 1994; Bhowmik and Doll, 1982). Putnam (1994) also cited some examples that inferred chemical toxicity of chickpea straw; they indicated that chickpea straw does not improve the soil as other legumes do, but instead "exhausts" and "scorches up" the land.

Comparing wheat yields between the *Flemingia* leaf and medic hay treatments at the high application rate it is apparent that wheat yield was about 2.5 fold higher with medic hay than with *Flemingia* leaf. This implies that the timing of nutrient availability affected yield and plant development. The tough leaf of *Flemingia* leaf remained in the soil after 1 month while only small amounts of decomposed litter of medic hay were observed. As a result, the wheat yields reflected the difference in the breakdown rate between the two residues.

ii) Soil carbon and nitrogen contents

Increased soil N and C in all treatment combinations compared to the control, are the result of residual N and C from the added residues. This agrees with numerous reports on effects of plant residue on the organic matter content of soils (Obatolu and Agboola, 1993; Toomsan *et al.*, 1993; Bouldin, 1988). The higher increase of soil N in treatments receiving *Flemingia* leaf, *Albizia* leaf and

medic hay reflect the combination of breakdown rate and N content in these residue. *Flemingia* leaf, *Albizia* leaf and Medic hay have N contents of 2.35, 2.69 and 2.23% respectively compared to those of 0.14 and 1.54% from wheat straw and chickpea straw. Higher soil N contents at the high residue application rate compared to the low rate are clearly due to the higher residue N additions. A similar observation was reported by Ladd *et al* (1983).

Unlike nitrogen, the residue C content does not relate to soil C content. The lower soil C in the treatments receiving chickpea straw and medic hay reflect the rapid decomposition rate of these residues which resulted in a greater loss of C as CO_2 into the air. Conversely, the slow decomposition rate of *Flemingia* leaf, *Albizia* leaf and wheat straw resulted in a low loss of C and a higher amount of C retained in the soil which can be observed from the high soil C in the soil receiving these residues. This agrees with the observation of Amato *et al.* (1987), who studied the decomposition of residues and found an inverse relationship between the decomposition rate and residual organic C in soil.

Budelman (1988) studied the decomposition of *Leucaena leucocephala*, *Gliricidia sepium* and *Flemingia macrophylla* and reported the lower decomposition rate of *Flemingia* leaf compared to the other two residues. Jenkinson (1981) has reported that for a range of crop residues (e.g. ryegrass, maize, wheat) about one-third of the input of plant C generally remains as organic residues after 1 year of decomposition in the field. Nevertheless, differences have been reported, which are more likely due to differences in plant properties.

b) The Second Wheat Crop

i) Wheat yield and nutrient uptakes

Wheat dry matter yields resulting from the high rate of residue application for the first and second crops are shown in Figure 4.8.

The residual effects of application of wheat straw at both rates still retarded wheat growth in the second crop and this negative effect was greater when the application rate was increased. At the low application rate, most of the major nutrients such as N, P, K and S were mineralised while at the high application rate all nutrients were immobilised. The explanation for this is as for the first crop and suggests that during the decomposition process the microorganisms were severely nitrogen limited. As a result, application of wheat straw to improve soil organic matter in this soil type would require inorganic fertiliser, particularly nitrogen, to overcome the short-term reduction in available nutrients. Inorganic fertiliser application would result in lower C:N ratio and provide more inorganic N in the soil which is readily available for the crop and microorganisms.

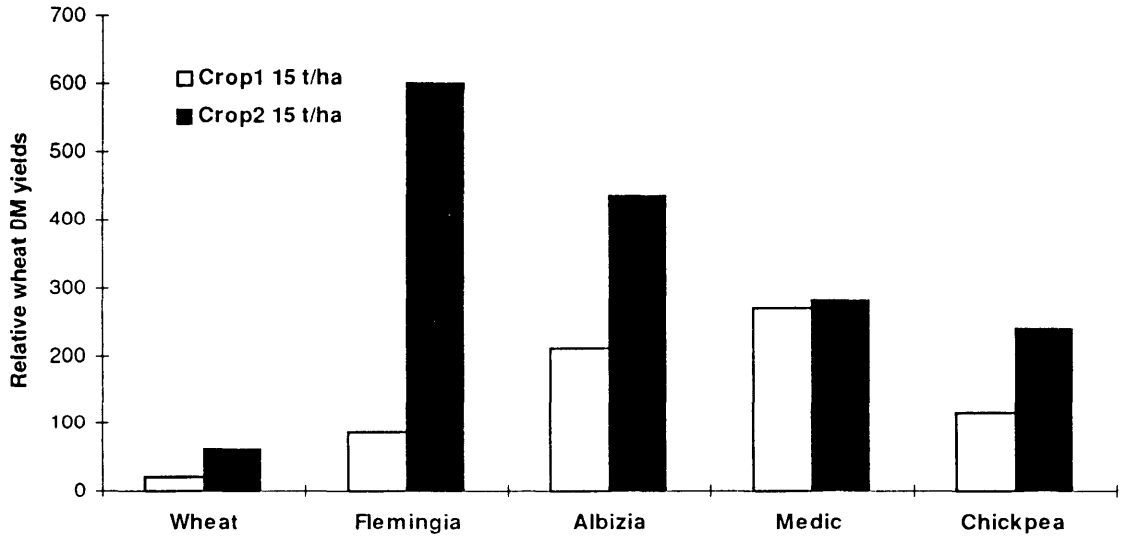

Figure 4.8 : Total dry matter yield of second wheat crop receiving different levels of different residues, relative to the control treatment (Control = 100).

Conversely, application of *Flemingia* leaf dramatically increased the wheat yield in the second crop. Although wheat yields at the low rate of *Flemingia* leaf application were comparable with the other residue treatments, at the high application rate *Flemingia* produced the highest dry matter yields compared to other residues and more than 6 fold more than the control treatment (Figure 4.8). After slow nutrient mineralisation or even immobilisation of some nutrients during the first crop, mineralisation proceeded rapidly during growth of the second wheat crop. These results show the slow decomposition and nutrient release rate of this residue, which confirms the result from the UNE perfusion apparatus. This agrees with Budelman, (1988, 1989) who reported the great persistence of *Flemingia* leaf and concluded that this residue would provide more durable cover and is a promising residue species for mulching, weed control and soil protection. Considering its poor performance in the first wheat crop suggests that in using this residue to improve soil organic matter a small amount of inorganic fertiliser would be required to compensate for the nutrients immobilised during the early stage of decomposition.

Application of *Albizia* leaf led to a similar observation as for *Flemingia* leaf, with a more than 4 fold increase in wheat yield compared to the control. At the higher rate, the increase in apparent nutrient recovery was reflected in the dramatic increase in wheat dry matter production in the second crop compared to the first crop (Figure 4.8). In the perfusion experiment *Albizia* leaf had a slightly faster decomposition rate compared to *Flemingia* leaf but a decomposition rate much lower than the medic hay and chickpea straw. This indicates that the breakdown rate of these two legume materials was initially low compared to the other legumes and that nutrient mineralisation occurred later than in these residues than with chickpea straw and medic hay.

Second crop wheat yields in the chickpea straw treatment at the low application rate, were decreased compared to the first crop (Figures 4.3 and 4.4). This indicates that the bulk of the nutrients had

been mineralised during the first crop due to the rapid breakdown rate of this residue. A similar observation was reported by Green and Blackmer (1995) who studied the mineralisation and immobilisation occurred during soybean (*Glycine max* (L.) Merr.) and corn (*Zea mays* L.) residue decomposition. In this study, wheat plants were grown under non-leaching conditions. The increase in wheat yields in the second crop at the high rate indicates the residual effect of nutrients mineralised during the first crop. Phytotoxicity limited wheat growth in the first crop and the released nutrients from this residue were readily available to the second crop.

At both rates of medic hay wheat yields in the second crop were slightly increased compared to the first crop indicating a residual nutrient effect, similar to that of chickpea straw. The wheat yields in the medic and chickpea treatments were comparable at each application rate although the yield from medic hay treatment was slightly higher. This is probably due to the higher content of major plant nutrients such as N, P and S in the medic hay and the apparent lack of any phytotoxic effects with medic hay.

ii) Soil carbon and nitrogen contents

The effect of adding residues prior to the first crop on soil C and N can still be seen after the second crop. The explanation for this is the same as presented for the first crop.

The reduction in soil C and N after the second wheat crop compared to the first is as expected. The N is probably lost through two pathways, i.e gaseous form and utilisation by the crop. The reduction in soil N is greater in the chickpea straw and medic hay treatments compared to the other residues. This is probably due to the greater loss through denitrification and some ammonia volatilisation. Volatilisation of ammonia can occur when easily decomposable and nitrogen-rich plant material decompose in the absence of soil (Floate, 1970).

The decomposition of residue C and loss as CO_2 is the reason for the loss of soil C. This is shown by lower content of soil C in the chickpea straw and medic hay treatments compared to the slow breakdown residues such as *Flemingia* leaf, *Albizia* leaf and wheat straw. Soil C content was comparable between high and low application rates in the chickpea straw and medic hay treatments. However, for the slow breakdown residue group, the C content of the soil was slightly higher with the high rate of residue addition than with the low rate. At the high rate of addition, undecomposed residue of *Flemingia* leaf, *Albizia* leaf and wheat straw could still be observed after the second harvest, while no undecomposed residue was observed from chickpea straw and medic hay treatments. However, the increase in soil carbon content was not in proportion to the residue added. This result supports the concept of Pinck and Allison (1951) who concluded that the percentage of carbon released when plant material was incubated with soil, compared to a soil alone control, was independent of the quantity added if the carbon addition did not exceed 1.5% of the dry weight of the soil and if decomposition was allowed to continue for at least three to six months. In the present study, residue application at the high rate added up to 1.3% of soil dry weight with the duration of the experiment of about 6 months.

4.4.3 Summary

Strong relationships were found between carbon released from the perfusion study and dry matter yield from the pot trial, and between N release and dry matter yield. This indicates that the plant residue quality assessed by the perfusion technique can be used to predict the outcome of using residues as soil amendments.

The generally good correlation between the results from the perfusion study and the pot trial indicates that the perfusion technique described in this experiment could be used for screening a wide range of crop residues and leaf litters under a standardised set of conditions, without the complication of differences in soil biota or deciding on the best forms of chemical analyses to compare residue. This would allow a ranking of relative breakdown and nutrient release rates. Inoculation of the $CaCl_2$ with soil solution extracts could be used to investigate the effect of different soil microbial populations on breakdown and nutrient release rates.

The affects of plant residue type on soil C and N, and on nutrient mineralisation, as described in this study, can be only used as general indicators because it was not possible to measure the proportion of the residue carbon and nutrients, such as N and S, contained in each part of the soil-plant systems. The fate of residue C and nutrients can only be resolved by using isotopes as tracers. For instance, using plant material labelled with radioisotopes, such as ^{14}C and ^{35}S, or using stable, isotopes such as ^{15}N and ^{13}C. This information will enhance our understanding of residue C and nutrient dynamics in agricultural systems, which is very important to develop better management of residue systems to improve soil organic matter.