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NUTRIENT MANAGEMENT STRATEGIES FOR SUSTAINABLE CROP PRODUCTION IN ACID SOILS

The Role of Research using Isotopes

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NUTRIENT MANAGEMENT STRATEGIES FOR SUSTAINABLE CROP PRODUCTION IN ACID SOILS: THE ROLE OF RESEARCH USING ISOTOPES

ABSTRACT

Malaysian agriculture thrives on 6.3 million hectares of land, mainly on highly leached acidic soils. These soils are inherently infertile due to nutrient deficiencies, especially of N, P, K, Ca and Mg, and/or heavy metal toxicities, mainly Al and Mn. Correcting these constraints with proper soil management measures can yield productive and sustainable farming systems. Improved management procedures in these systems must emphasize greater reliance on organic nutrient sources and the judicious use of inorganic fertilizers. This is especially for N and P, as overcoming deficiency in these nutrients usually generates the greatest improvement in crop yields.

Although there is fairly good understanding of the management of inorganic N and P fertilizers for crop production in acid soils, much less information is available on the management of organic forms of these nutrients, while data on interactions of the two forms is very much limited. Understanding the underlying processes in these strategies using conventional methods is most often difficult, but isotopes have been found to be useful in unraveling the mechanisms associated with nutrient availability, fertilizer use efficiency, losses and residual effects, as well as the role of legumes in farming systems. Also isotopes are important for monitoring soil moisture movement and availability, and in identifying varietal differences in nutrient use efficiency. Radioactive isotopes such as ^{32}P, ^{33}P, ^{35}S and ^{3}H have been used in studies on fertilizer use efficiency, nutrient management, root activity and water availability, whilst stable isotopes (^{15}N, ^{13}C) can provide valuable information on functions of ecosystems, organic matter dynamics and other N and C transformations.

Soil-plant studies conducted in Malaysia using stable and radioactive isotopes started in the mid 80's with the assistance of the International Atomic Energy Agency (IAEA) and the Malaysian Institute for Nuclear Technology Research (MINT). Some of the relevant findings and their links to the development and implementation of sustainable land management strategies for crop production are highlighted. The importance of isotope techniques in the validation of routine analytical methods and the identification of nutrient transformation mechanisms as well as the future prospects for improved environmental management are also highlighted.

INTRODUCTION

Malaysian agriculture thrives on highly weathered acidic soils, which are inherently infertile as a result of nutrient deficiencies, especially of N, P, Ca, Mg and Mo and/ or heavy metal toxicities, mainly Al and Mn. Correction of these disorders is often difficult, but failing to address them exacerbates the problem through further nutrient depletion and acidification. On the other hand, correcting these constraints with proper management procedures can result in more productive and sustainable farming systems. Although there are many problems affecting crop production in acid soils, the major limiting factors in most cases are deficiencies in N and P, and overcoming these problems normally generates the greatest improvements.

Improved management of these soils must involve adaptation of farming systems to greater reliance on organic nutrient sources in combination with the judicious use of inorganic fertilizers, especially P. Sustainable N replenishment strategies can rely on biological N^2-fixing processes, with limited need for chemical fertilizers. A similar approach is not possible for P. Therefore, improved P management strategies will continue to rely, to a much greater extent on the use of fertilizers.

Malaysia has been extremely successful in the agriculture business, being the leading producer of palm oil and pepper, and the third rubber producer in the world. The plantation industry strives to maintain its competitiveness through highly cost effective agronomic practices, which are both sustainable and environmentally sound.

This lecture will highlight some of the research undertaken in the sustainable management of acidic soils, through the use of radioactive and stable isotopes, that are relevant to the agriculture industry in Malaysia.

BASIC CONCEPTS IN USING ISOTOPE TRACERS

The first extensive use of isotopes as tracers in plant nutrition studies was in the 1940s. The radioactive isotope of P (^{32}P) was used to study the utilization of P fertilizers by various crops in a series of greenhouse experiments (Dean *et al.*, 1948) and field trials (Nelson *et al.*, 1948). Essentially, a tracer was incorporated into the fertilizer material or the nutrient source of interest, and the amount of tracer taken up by the plant was measured directly. The same procedure can be applied to stable isotopes such as ^{15}N.

An ideal tracer should have exactly the same behavior as the species being traced, and should have no effect on the components of the material in the system under study. Since tracer detection is very sensitive, the amount added in the experiment is usually very small compared to that already present in, or added to, the system. Thus there is very little disturbance of the system. Examples of isotopes useful in soil plant studies is shown below (Table 1).

Table 1: Examples of isotopes useful in soil plant studies (IAEA, 2001).

Elements	Most bundant isotope	Tracer isotope	Characteristics	Typical applications
Nitrogen	^{14}N	^{13}N	R, T½ = 10 min β (1.2 MeV) and γ (0.511 MeV)	Limited due to short half-life. Very short term studies on N^2 fixation, denitrification
		^{14}N	S, natural abundance = 99.634% $^{15}N/^{14}N$ ratio	N-14 enriched (N-15 depleted) materials for single season fertilizer use efficiency studies
		^{15}N	S, natural abundance = 0.3663% $^{15}N/^{14}N$ ratio	Fertilizer N use efficiency, biological N fixation, N balance, N transformation in soils, N availability from organic materials, animal nutrition studies
Phosphorus	^{31}P	^{32}P	R, T ½ = 14.3 d β (1.71 MeV)	Fertilizer P use efficiency, residual P studies, exchangeable P in soils, root activity pattern of crops, root distribution in soils, agronomic evaluation of phosphate rocks, residual P fertilizer availability
		^{33}P	R, T ½ = 25 d β (0.248 MeV)	Root autoradiography, diffusion in soils, double labeling for root activity patterns, fertilizer P use efficiency
Potassium	^{39}K	^{40}K	R, T½= $1.3\ 10^9$ y β (1.3 MeV) γ (1.46 MeV) Natural rdioisotope Natural abundance = 0.0118%	Exchangeable K in soils
		^{41}K	S, natural abundance = 6.77%	Potentially useful
		^{42}K	R, T ½ = 12.4 h β (3.5 and 2.0 MeV) γ (1.52 MeV)	Ion uptake mechanisms. Limited because of short half-life

One of the earliest experiments carried out on urea as a source of N for sweet corn grown on an acid soil at UPM was focused on identifying the optimum time of application. One rate of N (105 kg N ha^{-1}) applied once at planting was compared with three split applications of 35 kg N ha^{-1} at planting, silking and grain formation as follows:

Table 2: Treatments used to determine time of fertilizer N application to corn.

Treatment No.	Planting	Silking	Grain formation
T1	105*	0	0
T2	35	35*	35
T3	35	35	35*

* ^{15}N labeled urea

At harvest, it was found that only 15.3% of the N applied at planting was utilized by the corn crop, whereas in the three split applications, the N given at silking was more efficiently utilized at 28.3% compared to 19.3% obtained when it was applied at the grain formation stage (Zaharah *et al.*, 1989).

In phosphate studies, ^{32}P carrier-free materials, i.e. containing only ^{32}P atoms, are normally added as tracers. They can also be used for labeling commercial P fertilizers such as superphosphates during manufacturing or for preparing ^{32}P labeled solutions of known concentration for use in greenhouse and field experiments. Labeled phosphate solutions of high specific activity are also commercially available on request.

In the case of natural fertilizer sources like phosphate rocks (PR), direct labeling with P isotopes is not possible because of the changes induced in their physical and chemical characteristics during the procedure. Therefore, techniques based on reverse isotope dilution have been widely used to evaluate the availability of P from PR sources to plants (Larsen, 1952; Russell *et al.*, 1954; Sharpley, 1987; Zapata and Axmann, 1995; Kato *et al.*, 1995).

ISOTOPIC TECHNIQUES USED IN FERTILIZER EVALUATION STUDIES

An overview of isotope techniques used for evaluating nutrient availability from fertilizers, in particular ^{32}P isotope, to crops is made below.

a) Direct neutron irradiation of PR materials

This technique was first used by Fried and Mackenzie in 1949, and involved the incorporation of ^{32}P by direct activation of phosphate compounds in the PR through thermal neutron irradiation in a nuclear reactor, and the irradiated material was used as a labeled source in the evaluation of P uptake from PRs. It has been shown, however, that this method has serious drawbacks such as changes in the PR structure, the formation of non-orthophosphate ^{32}P components and of radioisotopes in the PR matrix, which complicate the determinations (Mackenzie and Borland, 1952). Hence this method is not suitable for evaluating PR sources and is therefore no longer in use.

b) Direct labeling of P and N fertilizer

When a phosphate fertilizer is applied to a soil, two sources (soil and fertilizer) that can provide P for a growing plant will be present in the soil. If the P from the fertilizer is labeled with an isotope of P (^{32}P or ^{33}P), it is possible to differentiate between soil- and fertilizer derived P in the plant. Thus, the amount of P taken up from the labeled P fertilizer can be measured directly. This direct method is used to compare several P fertilizers that can be labeled during their manufacture – this includes superphosphates, ammonium phosphates and nitrophosphates (Fried, 1954). The efficiency of P fertilizer management practices like timing, placement, combination of P sources etc, can also be determined using ^{32}P labeled fertilizers. The same procedure can be used for N fertilizers that can be abeled with the stable isotope ^{15}N. (FAO, 1980; Zapata, 1990; IAEA, 2001)

c) Methods based on isotope dilution

Natural fertilizer materials such as phosphate rocks and organic fertilizers cannot be labeled with an isotope in a similar manner as soluble phosphates and nitrogen fertilizers. For these materials, a reverse dilution method is used. Depending upon the system and experimental conditions used for equilibration of the isotope tracer, the measures of availability have been designated as E (Exchangeable P), L (Labile P) and A (Available P) values (Russell *et al.*, 1954; Fried, 1964; Larsen, 1967). These values may be computed using the following equation:

$$\text{A, L or E} = \frac{S^1X}{S^2} - X \qquad (1)$$

Where S_1 is the specific activity of the added tracer, X the amount of added carrier, and S_2 is the specific activity of tracer in the equilibrium solution.

i. A-value technique: The A-value represents a quantitative measure of the availability of a soil nutrient in terms of a standard fertilizer. The basic assumption is that when two or more sources of a nutrient are present in the soil, the plant will absorb from each of these sources in direct proportion to the respective quantities "available" to the plant. The amount of available (A-value) nutrient in the soil can be determined in terms of a standard, provided that the proportion of the nutrient in the plant derived from this standard is determined. This is done by using an isotopically labeled fertilizer (standard), which will give a direct measurement of the proportion of the nutrient derived from the standard fertilizer (Fried 1954, 1964). This method was first proposed by Fried and Dean (1952) and later developed at the IAEA laboratory, Seibersdorf (Broeshart, 1974; IAEA, 1976; Zapata *et al*., 1986; Zapata and Axmann, 1995).

The use of the "A-value technique" to evaluate PR products involves growing a test crop in a soil to which a ^{32}P labeled fertilizer is applied in a couple of treatments, with or without the PR or any other unlabeled P source. In order to calculate the A-value, the applied rate of each of these fertilizers must be known.

- Treatment 1 (standard): The crop is confronted with two P sources - Soil P + ^{32}P labeled fertilizer. From this treatment, the A-value of the soil can be determined. Since uptake by the plant is directly proportional to the available supply measured in the same units, the following relationship can be written:

$$\frac{PdfF}{AF} = \frac{Pdfs}{As} \qquad (2)$$

Where PdfF is the fraction of P in the plant derived from the ^{32}P labeled fertilizer, AF the amount of P supplied as ^{32}P- labeled fertilizer, Pdfs is the fraction of P in the plant that was derived from the soil, which is equal to 1-PdfF, and As is the A-value of the soil expressed in terms of equivalent units of the applied ^{32}P fertilizer (standard).

- Treatment 2: Soil + labeled fertilizer + Unlabeled PR.

From this treatment, the combined A-value of soil + PR can be estimated. The PR under study should be added at a P rate sufficient to supply the available amount of P, which results in a significant dilution of the radioisotope content in the plant, i.e a decrease in Pdff in the plant. In this treatment, the plant will have three sources of P (soil, ^{32}P labeled superphosphate and P from PR). Thus in this case, the following relationship can be written:

$$\frac{PdfF}{AF} = \frac{Pdfs}{As} = \frac{PdfPR}{APR} \qquad (3)$$

And

$$\frac{PdfF}{AF} = \frac{1-PdfF}{As + APR} \qquad (4)$$

Where PdfF is the fraction of P in the plant derived from ^{32}P labeled superphosphate, and AF the rate of P applied as labeled superphosphate. The fraction (1-PdfF) represents P in the plant that was derived from both the soil and PR.

As the amount of available P in the soil (As) is calculated from equation (2), APR can be obtained by subtracting As from (AS + APR). Since the P content and the amount of PR added are known, then the substitution ratios can be calculated i.e. the amount of PR that supplies the same quantity of P as the superphosphate standard under the experimental conditions (Zapata *et al.*, 1986; Zapata, 1990).

The same procedure can be used to evaluate nitrogen fertilizers available in the market or other organic fertilizers.

ii. L-value technique: The L-value is defined as the amount of labile P (in the soil and soil solution) that is exchangeable with ^{32}P labeled orthophosphate ions added to the soil, as measured by a plant growing in the equilibrated soil. The main difference with the E value is that S_2 is the specific activity of ^{32}P present in the plant. Table 3 shows the use of L-value for evaluating the relative agronomic effectiveness of natural PR sources applied to a tropical soil with oil palm seedlings under nursery conditions. The index, relative agronomic efficiency, was estimated by the following relationship:

$$(L_F - L_o) \ / \ P \text{ application rate} \qquad (5)$$

where L_F is the L value of the fertilized treatment and L_o is the L value of the treatment without P.

The PR sources tested were obtained from the local fertilizer Market and their agronomic effectiveness was compared to that of water soluble TSP. So Agronomists can choose the most economical P source for their requirements.

Table 3: Effect of PR application on available P (L value) for oil palm seedlings grown on Rengam series soil and the relative agronomic efficiency of the P fertilizers tested (Zulkifli *et al.*, 1998).

Treatments	L-value (mg P/kg)	Relative Agronomic efficiency (%)
Without P	22.4	0.0
N.Carolina PR	1238	54.3
China PR	491	20.9
Tunisia PR	850	38.3
Moroccon PR	592	25.4
Christmas Island PR	357	15.3
Jordan PR	729	31.3
Triple superphosphate	2401	100.0

iii. Reverse isotope dilution technique

In this technique, a ^{32}P carrier-free solution or one with carrier and a high enough specific activity is added to the soil and allowed to equilibrate with the soil P under adequate moisture conditions to label the pool of available soil P. The change in specific activity of the test plant is measured in the presence and absence of the PR being evaluated. The magnitude of decline in specific activity in the crop caused by the presence of a PR source (due to the dilution of the available soil P by P released from the applied fertilizer, PR in this case) relative to that of the treatment without PR represents the P that was taken up from the PR (Zapata, 1990). In order to apply this technique properly, it is required that i) the radiotracer effectively labels the pool of soil P that is available to the plant, ii) the rate of movement of the tracer from the pool of available soil P to other soil P pools is not greatly affected by the treatments and iii) the plant effectively samples the available soil P. The fulfillment of these requirements has led to adjustments / modifications of the technique, in order to be able to assess the P supplied from PR sources in tropical and subtropical soils with very low P status and, in many cases, very high P sorption capacity.

In the treatment standard where no unlabeled P material was applied, the system is considered to have only one source of P, i.e. the pool of available soil P that was labeled by isotopic exchange during the initial incubation period. In the fertilized treatment, the unlabeled P material (PR) is added to the labeled soil, and the P derived from PR will dilute the specific activity (sa) in the plant. The higher the availability of P from the PR source the greater the dilution, and this results in much lower sa in the plant. Therefore, the percentage of P from the unlabeled material can be calculated using the isotope dilution concept, where:

$$\%\ \text{PdfPR} = \left[1 - \frac{\text{sa plant with PR}}{\text{sa plant without PR}}\right] \times 100 \qquad (6)$$

The amount of available P in the labeled soil or AL can then be calculated in terms of PR equivalent units from the following equation:

$$\frac{\%\ \text{PdfPR}}{\text{P rate as PR}} = \frac{\%\text{PdfL}}{\text{AL}} \qquad (7)$$

Chien *et al.* (1996) employed this method to determine the enhancement effect in the uptake of P by maize and cowpea when concentrated Florida PR (CFPR) was applied together with TSP (Table 4).

Table 4: The average increase in P uptake by maize and cowpea from Central Florida PR (CFPR) as enhanced by triple superphosphate (TSP) (Chien *et al.*, 1996).

P Source	P uptake from CFPR (mg/pot)	
	Maize	Cowpea
CFPR alone (A)	2.11	1.93
CFPR + TSP (B)	5.99	3.31
(B) – (A)	3.88	1.38
(B-A)/(A) %	184	72

Similarly, Zaharah and Bah (1997) utilized this technique to assess the effect of the addition of several green manures on the uptake of P by maize plants from TSP and various PR sources in a Typic Hapludult. Also, in evaluating the effect on P uptake by setaria grass in similar circumstances (Table 5). The Main differences were due to the PR sources with little effect from the green manures.

Table 5: P uptake by Setaria grass from TSP and various PR sources (mg/pot) grown on Bungor series soil, as affected by green manure additions.

P source	Gliricidia sepium	Calopogonium caeruleum	Imperata cylindrica	No GM
TSP	68.5	53.0	75.0	25.6
APR	62.6	60.2	77.3	18.5
NCPR	59.4	62.0	75.5	44.3
CPR	68.5	46.9	75.5	26.4
No P	65.5	41.6	68.7	0.00

The above results proved the importance of the role played by organic matter in improving nutrient use by crops in our soils. The use of *Imperata cylindrica* as a mulch significantly improved P utilization by crops irrespective of the P source used. These findings have to be conveyed to local farmers so that such management methods could be incorporated in their cultural practices.

In an earlier experiment, we evaluated various P fertilizers available in the local market for their effectiveness in sweet corn production. This was measured in terms of total P taken up by the crop with time. However, evaluating the P sources in terms of yield and P proved inadequate and showed no differences in their effectiveness (Table 6). But with the use of ^{32}P marked differences in their effectiveness as a source of P for sweet corn became apparent (Figures 1 and 2).

Table 6: Sweet corn yield and total P taken up by sweet corn as affected by the addition of 100 kg P/ha (Zaharah *et al.*, 1994).

Phosphate Fertilizer Source	Yield (kg/ha)			Total P taken up (kg/ha)		
	CROP 1	CROP 2	CROP 3	CROP 1	CROP 2	CROP3
TSP	7357 a	7767 a	6640 a	14.43 a	19.22 a	15.34 a
NCPR	8824 a	6685 a	6742 a	15.11 a	22.71 a	16.27 a
CPR	8139 a	7152 a	6728 a	13.27 a	20.12 a	16.16 a

Figure 1: Percentage of P derived from fertilizers.

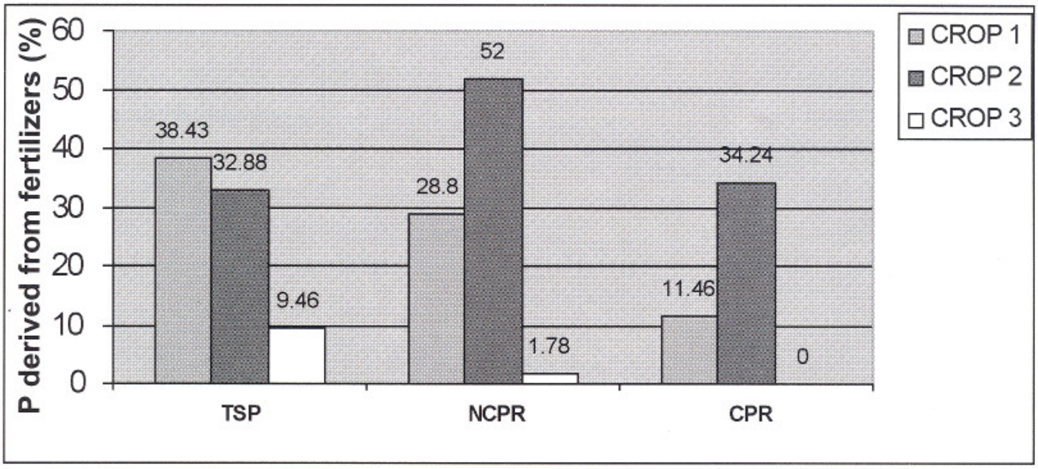
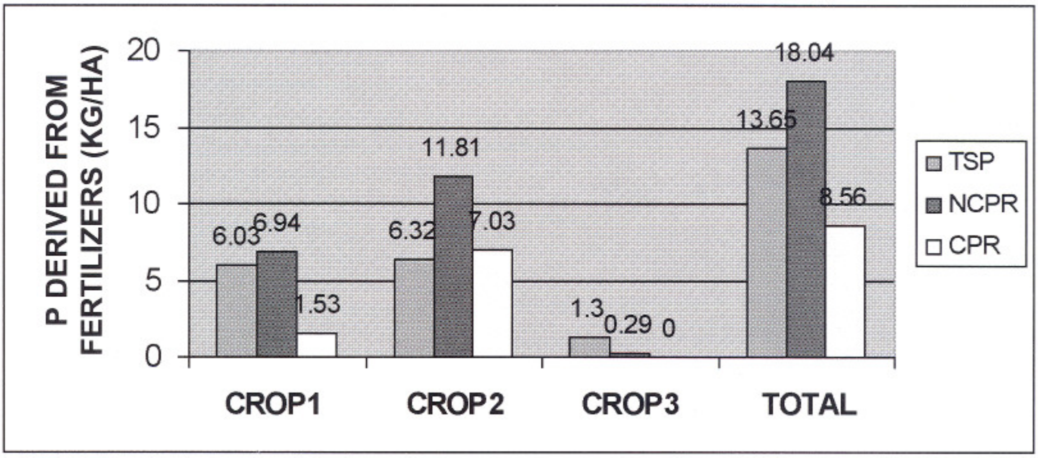


Figure 2: Total P taken up from fertilizers (kg/ha) (Zaharah *et al.*, 1994).



The same procedure was developed to measure N fixation by legumes, where both the legume, which is an N fixing crop (F) and a non-fixing reference (NF) crop are grown in a soil fertilized with enriched ^{15}N fertilizer (organic or inorganic). This method relies on the differential dilution in the plant of ^{15}N-labeled fertilizer by soil and fixed N (McAuliffe *et al.*, 1958; Fried and Broeshart, 1975; Fried and Middelboe, 1977). It provides an integrated measurement of the amount of fixed N accumulated by a crop over a growing season. The uptake of ^{15}N enriched fertilizer added to the soil will result in a $^{15}N/^{14}N$ ratio greater than 0.3663% within the plant. The extent of this increase is a reflection of uptake of the labeled ^{15}N fertilizer. A decrease in the atom % ^{15}N excess of the fertilizer N within the plant is an indication of the extent to which the plant took up N from other unlabeled sources, such as from the air (IAEA, 2001)

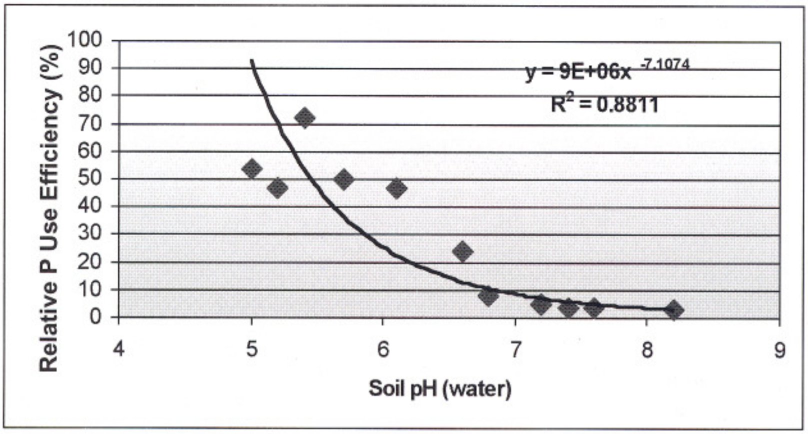
Our studies carried out at UPM to evaluate different species of N-fixing trees that can contribute biomass as a source of N to crops is shown below. It is evident that *P. falcataria* fixed the greatest proportion of N from the atmosphere at all stages (Table 7).

Table 7: Percentage of N2 fixed by N-fixing tree legumes at different ages

Tree species	4 months	6 months	30 months
P. falcataria	43.39 a	86.04 a	87.92 a
L. leucocephala	20.04 b	64.64 b	59.38 b
G. sepium	25.21 b	71.03 b	20.05 c
A. mangium	27.89 b	63.24 b	82.26 a
P. speciosa	10.12 c	0.00 d	0.00 d

Using the dilution technique, Morel et al (1986) conclusively demonstrated that whatever the initial P status of the soil, the use of ^{32}P labeled fertilizers in glasshouse and field experiments provides precise information on the effective short term utilization of P compounds. Using this method, they were also able to show the effect of soil pH on fertilizer P use efficiency (Figure 3).

Figure 3: Soil pH effects on P use efficiency of PR (Morel et al., 1986).



The immediate and residual P uptake from phosphatic fertilizers by crops has been measured by a dual labeling technique using ^{32}P and ^{33}P (Morel and Fardeau, 1989). Similarly, the effect of P rate and solubility of P fertilizers as well as the level of available soil P on P uptake from fertilizers has also been studied using ^{32}P labeled fertilizers. The application of the isotopic techniques described earlier requires the conduct of greenhouse and field experiments to determine the actual PdfF values of the various P fertilizers being studied.

Fardeau and Jappe (1988) and Fardeau et al (1991) have postulated a laboratory method to study the dynamics of soil P using radioisotopes. This method is based on ^{32}P isotopic exchange kinetics of P ions in a soil suspension (1:10 soil:water ratio) and determines the intensity, quantity and capacity factors in the system (Morel and Fardeau, 1991). Intensity factor (I) is the solution P concentration of the soil-water suspension (Cp). Quantity factor (Q) is the quantity of isotopically exchangeable phosphate ions in the soil solution at time t.

During the first minute of instantaneous exchange, it is equivalent to the E1-value. Capacity factor describes the ability of the soil to maintain the intensity factor constant when P is added to or removed from the soil. It is the ratio of isotopically exchangeable P at one minute (E1) to the P concentration in soil solution (Cp). This technique has been utilised to study P dynamics in some temperate (Sen *et al.*, 1988) and tropical (Salcedo *et al.*, 1991) soils. The effect of the supporting solutions used in preparing the soil suspensions on the value of the isotopically exchanged phosphate has also been studied (Morel and Torrent, 1997).

Based on the kinetics of isotopic exchange, Fardeau (1993) proposed a multi-compartmental model wherein total soil P can be divided into five pools or compartments according to their rates of exchange with a central pool of immediately available P. The most important pool is the central pool containing free P, i.e., P in solution plus P sorbed on the solid phase but having the same mobility as solution P. The P present in this pool (Pool E_1) can be exchanged with any P present in the other pools located in the solid phase of the soil. The other pools are: Pool A, the amount of P ions that is exchangeable with P in the soil solution from one minute to one day. Pool B is the amount of P ions, which is exchangeable with P in soil solution from one day to three months. Pool C is the amount of isotopically exchangeable P between three months to one year and pool D is the P ions that need more than one year to be exchanged with P in soil solution. These exchange times are based on physiological and agronomic principles (Fardeau, 1993). Thus, one day corresponds to the period of active uptake of P by a single root or root hair, and 3 months corresponds to the active uptake of P by the complete root system of annual crops.

This laboratory technique has been further extended to predict P bio-availability and the agronomic effectiveness of P fertilizers (Morel and Fardeau, 1991; Fardeau *et al.*, 1995). Also a P fixation index was developed from the parameters determined by the isotope exchange kinetics method (Frossard *et al.*, 1993). In the framework of the FAO/IAEA Co-ordinated Research Programme "Use of nuclear and related techniques for evaluating the agronomic effectiveness of P fertilizers, in particular rock phosphates" this technique has been further refined to evaluate P availability in soils of the tropics and subtropics with very low P status and high P sorption capacities, with and without P fertilizer additions (Zapata, 2000).

Results obtained by utilizing the ^{32}P isotopic exchange kinetic technique to measure P bioavailability from various PR sources and TSP in a Malaysian soil are shown below in Table 8.

Table 8: Isotopically exchangeable P parameters of the a Malaysian soil with the addition of 4mg P/kg of soil (Zaharah and Sharifuddin, 2002).

Parameter	Soil Alone	Soil+ NCPR	Soil+ TPR	Soil+ JPR	Soil+ MPR	Soil+ CIPR	Soil+ CPR	Soil+ TSP
Cp (mg P/L)	0.04 e	0.57 b	2.12 a	0.28 cd	0.41 bc	0.19 de	0.39 bc	165
E-1 min (mg/kg)	3.2 d	58 b	150 a	26 c	32 c	9.1 d	35 c	3836
Capacity factor (ml/g)	91 ab	101 a	70 c	90 ab	78 bc	48 d	86 abc	23
n	0.149	0.146	0.097	0.174	0.123	0.115	0.109	0.016
E-1 day (mg/kg)	10 d	161 b	281 a	85 c	77 c	18 d	75 c	2086
E-3mths (mg/kg)	0.34 c	11 a	9.9 a	6.0 b	12 a	0.93 c	0.34 c	1583
E-6mths (mg/kg)	0.12 d	23 a	17 b	14 bc	11 c	3.2 d	3.2 d	1732
E-9 mths (mg/kg)	0.5 c	46 b	91 a	43 b	34 b	6.7 c	6.0 c	614
Pool A (mg/kg)	9.6 d	160 b	279 a	84 c	76 c	18 d	75 c	1920
Pool B (mg/kg)	7.3 d	138 a	137 a	96 b	55 c	12 d	46 c	73
Pool C (mg/kg)	3 d	61 a	53 ab	46 b	24 c	5.3 d	19 c	22
Pool D (mg/kg)	63 e	3724 c	3610 d	3855 b	3927 b	4046 a	3942 b	1901
%Pdff (1-day)	0	86.2 b	93.5 a	68.6 c	76.1 c	17 d	75 c	99.8
%Pdff (3-months)	0	89.9 a	91.5 a	83.9 ab	77.9 b	18 d	73 c	98.7
%Pdff (6-months)	0	90.2 a	91.4 a	84.7 b	78.3 b	19 d	73 c	98.6
%Pdff (9-months)	0	90.7 a	91.7 a	85.7 b	79.3 bc	23 d	74 c	98.6
%Pdff (12-months)	0	90.4 a	91.4 a	85.5 b	78.7 bc	20 d	73 c	98.5

* Means in a row followed by the same letters are not significantly different at P=0.05

The use of ^{32}P isotopic techniques requires scientific and technical staff with adequate skills and expertise, and functional laboratory facilities to carry out the isotope measurements. In addition, the standard of the premises and personnel must comply with regulations set by the National Nuclear Energy Authority. The IAEA through its Department of Technical Co-operation assists developing Member States to enhance human and institutional capacities by providing equipment, expert services, scientific visits and fellowship training. Currently, a significant number of research groups distributed worldwide across developed and developing countries have the capacity to conduct such investigations.

In view of the growing concern and regulations imposed at the national level regarding the use of radioisotopes, it is difficult to carry out field experiments in many countries. Thus, most of the work with ^{32}P is done under greenhouse and laboratory conditions. The application of the isotopic techniques also has practical limitations in these experiments because of the more complicated logistics involved with a large number of experimental units due to the higher levels of radioactivity to be handled as well as the associated risk for radiation exposure (Zapata and Axmann, 1995).

ISOTOPES AS TRACER FOR ROOT ACTIVITY STUDIES

Root studies are an important component of crop management and improvement programmes. The roots of plants are responsible for water and nutrient uptake and therefore have profound influence on crop productivity. There are numerous methods for studying the root systems of plants, but most classical methods which involve visual observations and / or physical separation of roots aimed at determining the rooting patterns of crops do not provide information on root activity, growth and physiological responses to environmental factors. On the other hand. isotope techniques are unique tools, which provide quick and reliable information on the distribution pattern of active roots (Broeshart and Netsinghe, 1972).

The tracer methodology basically consists of injecting a suitable isotope to either the soil or the plant. In the plant injection technique, an isotope such as ^{32}P or ^{86}RB is injected into the stem of the plant and the pattern of root distribution is determined by taking soil cores containing roots, and measuring the radioactivity in the roots obtained. For the soil injection technique, a ^{32}P-labeled phosphate solution is injected into the soil at various positions (distances and depths). The radioactivity obtained in the plant samples will indicate the pattern of root activity at the various positions tested.

We have determined the active root distribution of matured oil palms and recommended that fertilizers be distributed evenly along palm rows rather than singly around each palm (Zaharah *et al.*, 1989).

Wan Rashidah *et al* (1998) used a similar technique for determining the active root zone of a commercial *Acacia mangium* forest plantation and rotan manau intercropped with rubber (Wan Rashidah *et al.*, 1997). They found out that at 3 months after field planting, the roots of *A. mangium* were still within the polybag area. However, after 9 months, the active roots had extended up to 150cm from the base, and they were concentrated within 370 cm in the upper 30 cm soil layer at 21 months after planting.

For rattan grown under rubber, the roots had grown horizontally about 150 cm away from the plant base at 3 years after planting. The greatest proportion of the roots was in the surface layers, concentrated at a distance of 50 to 100 cm from the plant. After 4 years, the roots had grown to more than 230 cm away from the plant base. Based on these results, it is apparent that broadcasting fertilizers either around the plant or along the inter-rows between rattan and rubber trees may lead to similar fertilizer utilization efficiencies.

USE OF ^{15}N IN STUDYING THE ROLE OF N-FIXING TREES

Trees also play a role in our agriculture, especially those that can fix atmospheric nitrogen. The potential soil fertility benefits of using leaves of tree legumes like *G. sepium* and *S. sesban* in agroforestry systems is well acknowledged by various authors (Wade and Sanchez, 1983, Zaharah *et al.*, 1996, Buresh and Tian, 1997, Kwesiga *et al.*, 1999). Mafongoya *et al* (1997, 1998) attributed the increase in maize grain yield under improved fallow management to the increase in soil inorganic N resulting from decomposition of the incorporated leaves and twigs.

Periodic pruning of above ground biomass (shoots) is a common management practice in agroforestry systems such as alley cropping, biomass transfer and improved fallow systems that integrate coppicing legume tree species. The practice does not only promote leaf biomass production and the recycling of N from the leaves, but it also induces root N turn over (i. e some amount of fine roots die, decompose and eventually mineralize and enhance soil inorganic N availability). Lehmann *et al* (1998) concluded from a review of the literature that the ratio of live-to-dead roots decreased when trees were pruned, suggesting root biomass shedding. Bashir *et al* (1998) reported that, about 20 to 50% of root residues from trees and shrubs in a tropical self regenerating fallow is converted to soil humus compared to only 10 to 20% of the above ground litter.

According to Palm (1995) and Giller et al (1995), crop nitrogen recovery from the incorporated prunings range between 10 to 20%. However, there is an indication that root N contribution to crops in agroforestry systems could be substantial since below ground biomass accumulation by tree roots can be considerably high, in the order of 3 to 6 Mg ha^{-1} year^{-1} (Sanchez, 1995). The mineralization rate of organic matter is one of the major factors that influence N availability to crops. Synchrony or asynchrony of N demand by the crop with N supply from an organic source also depends on the rate of mineralization and the timing of application of the organic source. A green house study by Mafongoya *et al* (1997) highlighted three important factors that influence mineralization of organic residues, namely: chemical composition (i.e residue quality), cultural practices (i.e placement, timing and rate of application) and soil properties. However, the environmental or microclimatic conditions (temperature and moisture) may override all the above factors. When physical and environmental factors are not limiting, residue quality plays a much more important role in regulating decomposition rate (Buresh *et al.*, 1998).

By using ^{15}N labeled leaves from *P. falcataria* trees, we established the rate of nutrient released by fresh leaves applied as mulch in a field experiment (Figures 4 and 5). Directly labeling the tree with ^{15}N labeled urea, and then reincorporating the labeled materials into the soil was also used to quantify the N contribution of the above- and below-ground biomass of a legume tree to an associated crop (Figure 6).

Figure 4: Changes in N released from surface applied *P. falcataria* leaves into the 10 cm soil layer over time.

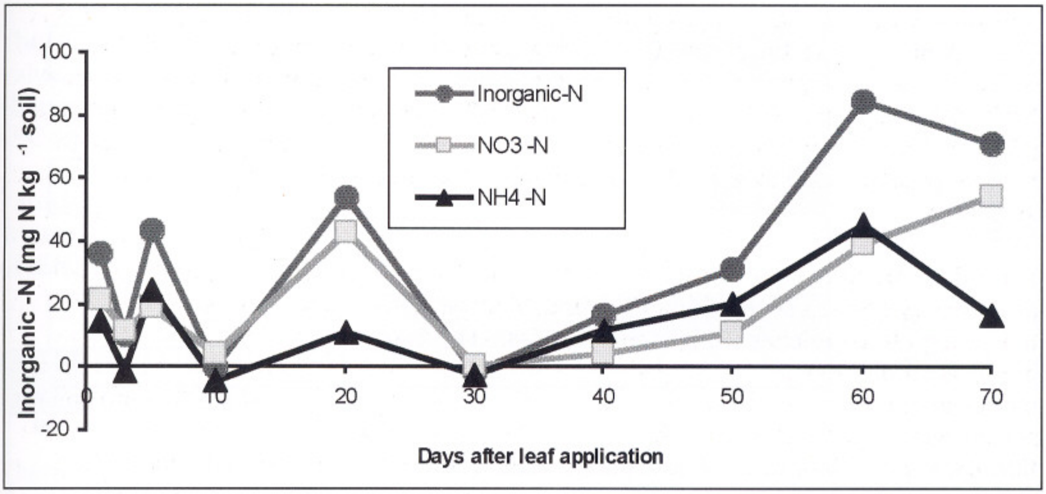


Figure 5: N in the 10-20 cm soil layer when *P. falcataria* leaves were placed on the soil surface.

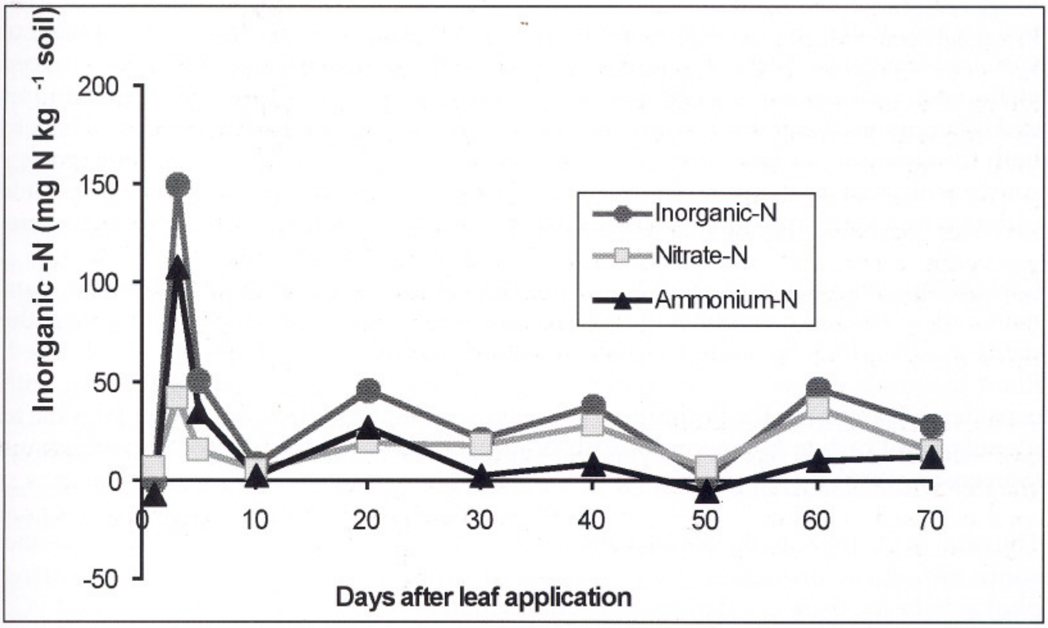
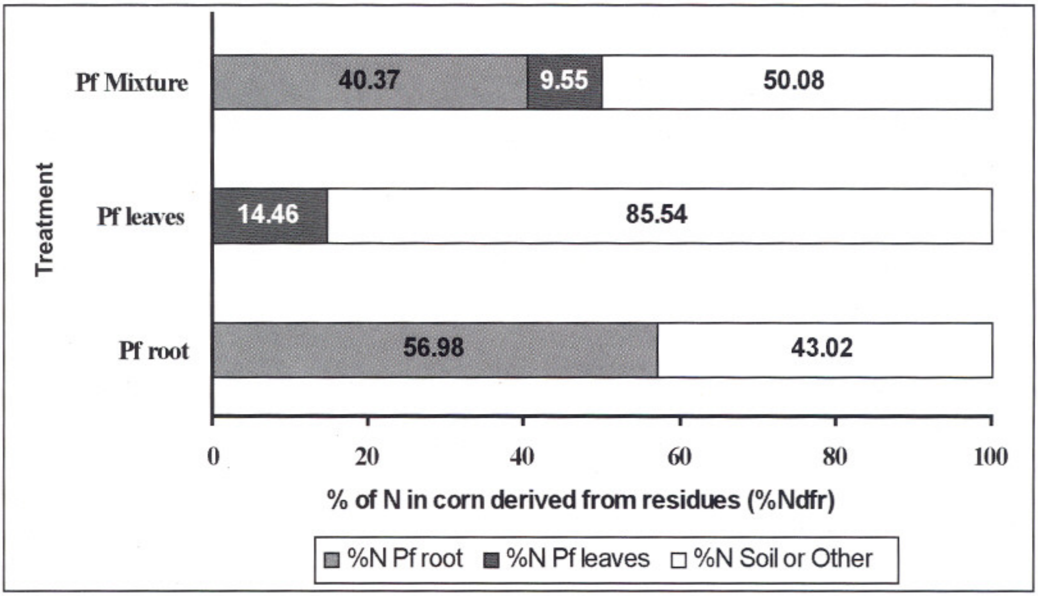


Figure 6: Nitrogen contribution by leaves and roots of *P. falcataria* to corn



^{13}C AND ^{14}C ISOTOPE STUDIES

Soil organic matter (SOM) studies are becoming increasingly important as the concept of sustainable agriculture gains prominence with attempts to produce enough food to feed an the ever-increasing world population. The use of agricultural residues, green manures and other organic amendments are some of the ways of increasing SOM which will benefit both the chemical and physical effects of the fertility of the soil. Carbon is the energy source which drives many of the nutrient cycles in the soil. A ready supply of accessible carbon is necessary for a continuous supply of soil nutrients for maintaining soil structure.

Carbon isotopes such as ^{13}C and ^{14}C are used for tracing the additions of different plant materials to the huge amounts of carbon already present in the soil, and to follow the pathways by which the added carbon contained in the plant materials are transformed. Plant materials can be labeled with ^{13}C or ^{14}C, and it is then possible to trace with considerable accuracy, the decomposition of added plant residues. It is even possible to identify plant carbon as it became incorporated into different fractions of the soil humus (Stevenson, 1986).

The ratio of ^{13}C to ^{12}C in the atmosphere can vary with altitude, latitude, temperature and some biological processes (Lefroy et al., 1995). When plants fix carbon during photosynthesis, there is a degree of discrimination between the amounts of ^{13}C and ^{12}C . This discrimination occur during the carboxylation step in photosynthesis, with greater discrimination against ^{13}C in C3 (Calvin cycle) plants than C4 (Hatch-Slack cycle) plants due to the greater discrimination in the primary carboxylation step of C3 plants, which results in a lower ^{13}C / ^{12}C ratio in C3 plants than C4. CAM (crassulacian acid metabolosm) plants show variable discrimination, but it is more often similar to C4 plants.

The ^{13}C / ^{12}C ratio is generally measured as d^{13}C. A C4 plant such as corn will have a d^{13}C value of ~ -12 %0 , whereas a C3 species like rice or wheat will be ~ -26 $\%_0$.. Thus every change in vegetation between C3 and C4 plants will result in a corresponding change in δ^{13}C value of SOM (Lefroy *et al.*, 1993).

Farquhar and Richards (1984) reported the use of carbon discrimination for studies on water use efficiency by plants. They use Δ ^{13}C values to determine the water use efficiency of different species.

FUTURE PROSPECTS FOR APPLICATION OF ISOTOPIC TECHNIQUES

There is considerable scope for further application of isotopic techniques to get a better understanding of the dynamics of soil nutrients and for evaluating the agronomic effectiveness of fertilizer sources in cropping systems of well-defined agro-ecological zones.

A key strategy for sustainable intensification of agricultural production is the identification of suitable plant genotypes adapted to particular soil and climatic conditions. For instance, in most tropical soils of the savanna agro-ecosystem, plant genotypes should be P-efficient and Al-tolerant. ^{32}P techniques are useful in the identification of these genotypes. Furthermore, the adapted genotypes should be evaluated in terms of their contribution to the overall productivity and sustainability of the cropping system. Most important for resource-poor farmers is that the combination of these adapted genotypes with the application of PR may be a cost-effective strategy to combat acidity and P deficiency in tropical acid soils.

Environmental issues related to the utilization of fertilizers and organic matter turnover in soils are also a matter of major concern, so isotope techniques can be used to accurately define levels of fertilizer and organic inputs in order to minimize losses from soils to surface waters and to help improve soil quality.

Several studies have also been conducted to evaluate the performance of routine soil chemical methods using the isotopic exchange kinetics as reference (Zapata, 2000). More recent studies focus on the mechanisms of the mobility of nutrient ions, i.e. the rate and extent of soil-solution transfer of nutrient ions in both nutrient-deficient and heavily fertilized soils (Morel *et al.*, 2000; Schneider and Morel, 2000).

In conclusion, it should be noted that isotopic techniques are important in plant nutrient and organic matter investigations. They provide more accurate and reliable information on various mechanistic studies of nutrients in soil and soil-plant systems. Therefore, they are essential for developing knowledge-based nutrient management practices for sustainable intensification of agricultural production and environmental protection.

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