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Effect of tissue phosphorus concentration on the mineralisation of nitrogen

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Abstract

Laboratory studies were conducted to investigate the effect of phosphorus concentration in residues of cowpea (*Vigna unguiculata*, L. Walp) and stylo (*Stylosanthes hamata*, L., cv Verano) on their rate of nitrogen mineralisation when incubated in a soil whose P status was deficient for plant growth. Residues with a range of P concentrations were obtained by applying varying rates of P to soil in which the plants were grown in the field or the glasshouse. Variations in P concentration of field- or glasshouse-grown residues were not accompanied by variations in other chemical components (C:N ratio, lignin and polyphenol concentrations). Both lignin and polyphenol concentrations were higher in the field-grown than in the glasshouse-grown residues. Lignin concentration was greater in cowpea than in stylo, but polyphenols were higher in stylo. Cowpea residues mineralised N less rapidly than stylo. N mineralisation from residues with low P concentration was consistently less than from those of higher P concentration; reduced mineralisation was observed for P concentration in the residues below 1.6 g kg^{-1}. When inorganic P was added to the residue-soil systems, N mineralisation from the residues was increased, though no interaction between the effects of adding inorganic P and P concentration in the residues was observed.

Introduction

Nitrogen (N) is the most limiting nutrient in many farming systems of the semi-arid tropics (Fox, 1978). We believe this to be particularly so in eastern Kenya. Yet despite the widespread N deficiency that limits the production of maize crops, the majority of farmers do not use N fertilisers (Muhammad and Parton, 1992). The only inputs of N are from the use of manure and from legumes that are commonly grown as intercrops with maize, but these sources provide insufficient N to meet the needs of the crops in this farming system. Since increasing production depends on a better supply of N, it is necessary to improve the utilisation of the traditional supplies of N and, where necessary, augment these with fertiliser (McCown et al., 1992).

The total input of N to the soil-crop system from crop residues is determined primarily by their total N content, but the contribution they make to the nutrition of crops depends on their rate of decomposition which varies with the nature of the residues. This quality aspect of the residues is often considered in terms of the carbon:nitrogen (C:N) ratio. Where C:N is high, N is immobilised by the microbial population. The C:N ratio of materials below which net mineralisation occurs is about 25 (Stevenson, 1986). Other quality aspects of the residues that have been shown to influence the rate of mineralisation are the contents of lignin and polyphenol (e.g. Fox et al., 1990; Palm and Sanchez, 1991).

Phosphorus (P) has been shown to affect N mineralisation from soil organic matter (Munevar and Wollum, 1977; Ryan et al., 1972; Stotzky and Norman, 1961), but has attracted little attention as a factor that might influence the rate of N mineralisation from crop residues. However, recent reports (Cheshire and Chap-

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man, 1966; Enriquez et al., 1993) indicate that P can be a factor in the decomposition of plant residues.

In as much as P deficiency restricts plant growth, the total N uptake varies with dry matter yield. However, plants grown under a P limitation also have lower P concentrations in their tissues. The hypothesis tested in this study is that the P concentration in residues influences the rate of N mineralisation. We report the results of incubation studies conducted in the laboratory using residues of stylo (*Stylosanthes hamata* L. c.v. Verano) and cowpea (*Vigna unguiculata* L. Walp accession number CPI67233) with a range of P concentrations resulting from the conditions under which the plants had been grown.

Materials and methods

Incubation procedures

Three experiments were carried out. For all experiments, the incubation units comprised a mixture of 20 g air dry soil (ground to pass a 1 mm screen), 20 g washed sand (0.8 mm diameter) and 1 g of residues.

In Experiment 1, N mineralisation was measured after 4 weeks incubation from three cowpea and three stylo residues that had been grown in the field. In Experiment 2, mineralisation was measured after 4 and 8 weeks, in separate incubation units, for four cowpea and four stylo residues grown in the glasshouse. This experiment also studied the effect of whether the residues were ground or unground, and the effect of added inorganic P (20 mg kg^{-1} of soil as NaH_2PO_4) for the residues with the lowest P concentrations. For these experiments, the soil-sand-residue mixture was placed in 150 mL plastic containers, measuring 4.5 cm in diameter and 10.5 cm in height. Water was added to bring the moisture content to 12 percent, approximating the field capacity of the soil, which was maintained during the incubation period by addition of water when necessary. The top of each container was covered with parafilm with three pin-holes to facilitate gaseous exchange and maintain an aerobic system. At the end of the incubation period, mineral N (and P for Experiment 2) was extracted from the mixture in each container.

Experiment 3 studied the time course of N mineralisation over 8 weeks using a leaching technique to ensure that accumulation of mineral N did not inhibit mineralisation (Harmsen and Schreven, 1955) for the field-grown residues, with and without the addition of inorganic P. The soil-sand-residue mixture was transferred into 100 mL leaching tubes. Mineral N initially present was removed by leaching with 100 mL of 0.01 *M* $CaCl_2$ in 20 mL increments, followed by 25 mL of a nutrient solution devoid of N and P (0.002 *M* $CaSO_4.H_2O$, 0.002 *M* $MgSO_4.H_2O$ and 0.0025 *M* K_2SO_4, similar to that used by Frankenberger and Albdelmagid (1985)), and for the plus P treatments a solution of NaH_2PO_4 supplying 40 mg kg^{-1} soil. After adjustment of the moisture content (60 cm H_2O vacuum), the tubes were covered with parafilm and placed in the incubator. Every two weeks, mineral N was recovered by leaching with 0.01 *M* $CaCl_2$, followed by addition of the nutrient solutions and re-adjustment of the moisture content before the tubes were returned to the incubator.

All experiments used a randomised block design with three replications and included controls (without added residues) along with the residue treatments. During incubation, each block was on a different shelf in the incubator which was maintained at 30 °C. Re-randomisation of treatments within a block was carried out weekly.

Soil

The soil used for the incubation studies was the surface 0–15 cm of a Tindall clay loam (Aldrick and Robinson, 1972) (Oxic paleustalf) from Katherine, Northern Territory, Australia. It contained 5 mg kg^{-1} bicarbonate extractable P using the method of Colwell (1963). For this soil type and extractant, the critical P concentration indicative of adequate P supply is 25 mg kg^{-1} for cereals and 10 mg kg^{-1} for stylo based pastures (Incitec Ltd, unpublished).

Crop residues

Residues with different P concentrations were obtained by growing cowpea and stylo on this P deficient soil with varying fertiliser P inputs chosen to cover the full range from deficiency to non-limiting P supply. The residues used in Experiments 1 and 3 were grown in the field at Katherine. The stylo was harvested 94 days after sowing (DAS) and cowpea 61 DAS. At the time of harvest, cowpea was mature and the pods and grain were not included in the residues. The leaf and stem components for both species were ground to pass a 1 mm sieve and stored in plastic containers prior to use.

The residues used in Experiment 2 were grown in pots in a glasshouse at the Cunningham Labora-

tory, Brisbane. Stylo was harvested 86 days DAS; cowpea was mature at the time of harvest (58 DAS). Leaf and stem components were used. A portion of the residues was ground as above; for the unground treatment, residues were cut into approximately 1 cm lengths prior to mixing with the soil and sand.

Analytical methods

In the residues, carbon was determined by a modified dry combustion procedure described by Carr (1973). Lignin and polyphenol contents were measured using the methods employed by Fox et al. (1990), namely the acid detergent fibre-permanganate method of van Soest and Wine (1968) for lignin and the Folin-Denis method of Burns (1963) for polyphenol extracted during five hours refluxing with water. Total N was determined by the salicylic acid modification of the Kjeldahl method described by Bremner and Mulvaney (1982). Total P, potassium and sulphur were determined as described by Johnson et al. (1985). Soluble P was extracted from the ground residues with deionised water by shaking for 16 hours (1:100 residue: water ratio) and determined using an inductively coupled plasma mass spectrometer (ICP-MS) (VS PLASMAQUAD).

Nitrate- and ammonium-N in soil were determined using extraction with 2 *M* KCl and the MgO-Devarda alloy distillation method of Keeney and Nelson (1982). P was extracted with 0.5 *M* $NaHCO_3$ using the method described by Colwell (1963). Where extractable P was measured (Experiment 2 only), a subsample of one tenth of the moist soil-sand-mixture was removed from each container to determine extractable P with the remainder being used for the extraction of mineral N.

Net N mineralisation was calculated as the difference in mineral N between the residue treatments and the appropriate control treatment without residues. To permit comparisons between the various residues, net mineralisation has been expressed as a percentage of the N added in the residues.

Results and discussion

Chemical composition of residues

Analytical data for the residues are set out in Table 1. For both species the field- and glasshouse-grown material had a range of P concentrations reflecting the P supply under which the plants had been grown. P deficiencies had occurred in both the field and glasshouse with reduced dry matter production where the lowest P concentrations were measured. The lowest P concentrations were in the field-grown material which also had a much smaller range of P concentrations than was present in the material from the glasshouse.

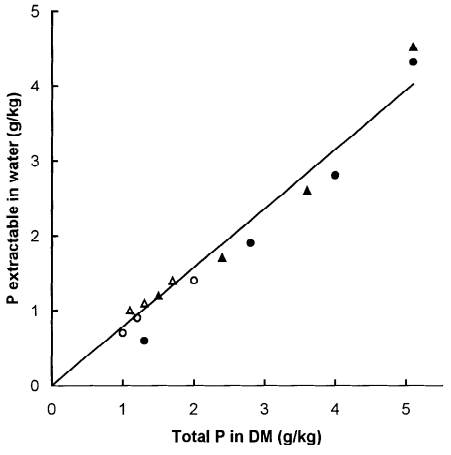

Figure 1. Relationship between phosphorus concentration in dry matter and water extractable phosphorus in stylo (Δ) and cowpea (○) residues. Solid symbols denote material grown in the glasshouse, open symbols the field grown material.

The variation in total P concentration of the residues was accompanied by variation in water-soluble P concentration (Figure 1). There was a close relationship between water-soluble P and total P concentrations, with the field and glasshouse materials for both stylo and cowpea fitting the same relationship. The linear regression did not have a significant intercept and indicated that about 75% of the total P was extractable with water. This value is similar to that found by Bromfield and Jones (1972) who reported 60–83% of the total P was water-soluble in ground samples of clover and phalaris, with most of it being inorganic.

Despite the range of P concentrations and the P deficiency experienced by some of the growing plants, there was little variation in the concentrations of N, C, S, K, lignin and polyphenols within species when grown under the same conditions (Table 1). The absence of major differences in N concentrations is important when we consider the N mineralisation exhibited by the various materials, since the residues were added on an equal dry matter basis. However, there were consistent differences between stylo and cowpea in lignin and polyphenol concentrations. Lignin was higher in cowpea than in stylo, whilst polyphenol was higher in stylo than in cowpea.

Table 1. Chemical composition (g kg^{-1} dry matter) of legume residues

Field grown							Glasshouse grown					
P	C	N	S	K	Lignin	Poly-phenol[a]	P	N	S	K	Lignin	Poly-phenol
Stylo												
1.1	410	27.0	1.5	27	53	21	1.5	29.4	1.3	33	49	17
1.3	410	26.5	1.1	25	52	25	2.4	29.0	1.2	30	46	18
1.7	410	26.9	1.2	29	53	23	3.6	28.9	1.4	32	46	19
							5.1	30.1	1.5	36	47	18
Cowpea												
1.0	430	27.2	1.4	30	62	19	1.3	29.8	3.1	36	56	12
1.2	430	27.2	1.3	23	63	18	2.8	28.7	2.0	33	55	12
2.0	440	27.2	1.5	27	61	19	4.0	28.9	1.6	32	54	12
							5.1	28.9	2.0	32	56	12

[a]Tannic acid equivalent.

For both species, lignin and polyphenol were higher in the field-grown than in the glasshouse-grown material.

Nitrogen mineralisation

Much less N was mineralised from the unamended soil than where residues were added. In Experiment 1, for example, 0.66 mg mineral-N (per incubation unit) was present in the control treatment after 4 weeks incubation compared with between 4.76 and 8.62 mg where residues were added. Most of the mineral-N that accumulated was present as nitrate, indicating that nitrification was not limiting.

The results from the three experiments are presented in Tables 2 and 3, which show the net N mineralisation expressed as a percentage of the N added in the residues. Grinding the residues (Experiment 2) resulted in only small increases in the percentage N mineralised, averaging 0.9% for the stylo residues and 2.4% for the cowpea residues with a least significant difference (LSD) of 0.9 (p = 0.05). Thus the results given for Experiment 2 have been averaged across the ground and unground treatments. For Experiment 3, Table 3 gives the percentage of residue N that was mineralised in each two week interval and the total during 8 weeks.

Time course of N mineralisation

The leaching technique used in Experiment 3 (Table 3) shows that the rate of mineralisation was fastest in the first two week period but continued, at a reduced rate, for at least 8 weeks. Comparison of Experiments 1 and 3, which used the same residues, shows that more N was mineralised in 4 weeks using the leaching technique, where N was removed every 2 weeks, than in a single 4 week incubation. In Experiment 2, there was only a small increase in N mineralised between the units that had been incubated for 4 or 8 weeks.

Effects of different residues

Mineralisation from cowpea residues was consistently lower than from stylo residues. Also the mineralisation from the field-grown residues (Experiments 1 and 3) was less than from the glasshouse-grown residue used in Experiment 2. The N concentrations in the field-grown residues were only slightly lower than in the glasshouse material and differed little between the cowpea or stylo residues from a common source. Differences in lignin and polyphenol concentrations of the residues, with respect to both the species and whether the residues had been grown in the field or glass house (Table 1), were not large when compared with the ranges of lignin and polyphenol reported by others (Fox et al., 1990; Palm and Sanchez, 1991) when establishing these as factors controlling mineralisation. The results do support the findings of Fox et al. (1990) that the rate of mineralisation decreases with increasing (lignin + polyphenol): N ratio. For these residues, however, it would seem that lignin had a greater influence on mineralisation than polyphenol. The lower mineralisation from cowpea residues corresponded with higher

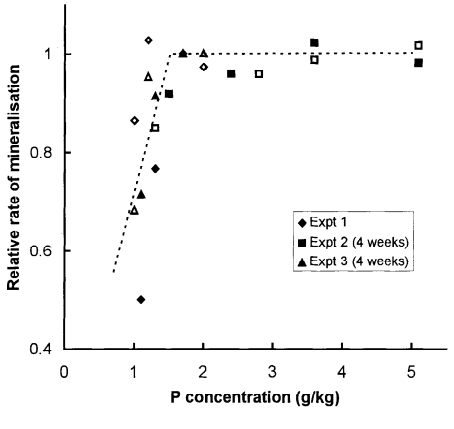

Figure 2. Relative rates of mineralisation of nitrogen from residues of varying P concentrations. Relative mineralisation is expressed as the quotient of measured mineralisation for each residue and the highest rate observed for the same type of residue in the same experiment. Solid symbols denote stylo, open symbols cowpea.

lignin concentrations, but polyphenol concentrations were lower in the cowpea residues than in stylo.

Effects of P concentration in the residues

The results from all three experiments show that the rate of N mineralisation was reduced significantly when the P concentration in the residues is low. The largest effect was observed for stylo in Experiment 1 (Table 2) where the proportion of N mineralised doubled when the P concentration increased from 1.1 to 1.7 g kg^{-1}. The effect was much smaller for the field-grown cowpea residues, one of which had the lowest P concentration of all the residues studied, though the same residue used in Experiment 3 exhibited a larger reduction in mineralisation. Experiment 3 (Table 3) showed that the effect of P concentration on N mineralisation persisted as mineralisation proceeded during the eight weeks of incubation.

The observed effect is likely to be manifested through the nutritional requirements of the soil microorganisms that are responsible for the decomposition of residues in soil. It raises the question whether there is some critical P concentration, C:P or N:P ratio in residues which signifies an adequate P supply to meet the needs of the microbial population and the synthesis of soil microbial biomass. Figure 2 shows the relative rate of N mineralisation from the stylo and cowpea residues in the three experiments plotted against the P concentration in the residues. To standardise between experiments, the data plotted are all after four weeks incubation. The range of P concentrations in the field-grown residues (Experiments 1 and 3) was not sufficiently wide to establish unequivocally that the rate of N mineralisation had reached a plateau with respect to increasing P concentration. Nonetheless, Figure 2 suggests that the rate of N mineralisation of residues is related to their P concentration. The linear response and plateau model fitted to these data indicates that N mineralisation is reduced when the P concentration in the residue is below 1.6 g kg^{-1}, which corresponds to a C:P ratio of 250.

Effect of added inorganic P

If P concentration in the residues can be limiting for mineralisation, there is presumably opportunity for the deficit in P requirement to be obtained from the soil, thereby removing the limitation. The soil used in this study was low in extractable P and is known to be deficient for plant growth. It is an unanswered question whether the effect of low P concentration in residues would have been greater in a soil of lower P status.

Where soil P status was improved by adding inorganic P, N mineralisation from residues was increased. In Experiment 2 (Table 2), N mineralisation from the residues with low P concentration was increased when inorganic P was added, though, in the case of the cowpea residues, not to the extent of matching mineralisation from the residues with higher P concentrations. The results from Experiment 3 (Table 3) indicate that adding inorganic P increased the total N mineralisation during 8 weeks irrespective of the P concentration in the residues. The supposition that the microbes might offset a limiting P supply from the residue by a greater contribution from the soil would be expected to result in an interaction between the effects of P concentration in the residue and adding inorganic P. However such an interaction was not significant ($p > 0.05$) for the results from Experiment 3.

Phosphorus mineralisation

Extractable P in soil following incubation with residues was measured only in Experiment 2. The control treatment, without added residues or inorganic P, assayed 10 μg g^{-1} of soil after 4 weeks incubation and 8 μg g^{-1} after 8 weeks. Where residues were added, extractable P decreased significantly ($p < 0.001$) between 4 and 8 weeks of incubation, with an average decrease across all treatments of 7 μg g^{-1}. Table 4 reports the increase

Table 2. Net nitrogen mineralisation, as percentage of nitrogen applied, from residues with varying phosphorus concentrations

Experiment 1		Experiment 2		
P concentration (g kg^{-1})	% N mineralised in 4 weeks	P concentration (g kg^{-1})	% N mineralised in 4 weeks	% N mineralised in 8 weeks
Stylo				
1.1	15	1.5	45	47
1.3	23	1.5 (+P)[a]	51	50
1.7	30	2.4	47	50
		3.6	50	52
		5.1	48	51
Cowpea				
1.0	16	1.3	31	34
1.2	19	1.3 (+P)	34	35
2.0	18	2.8	35	40
		3.6	36	42
		5.1	37	41
LSD (p = 0.05)	1.0		2.0	

[a] Inorganic P added.

Table 3. Net nitrogen mineralisation, as percentage of nitrogen applied, from residues with varying phosphorus concentrations during successive incubation periods, and as influenced by added inorganic phosphorus. LSDs, for each incubation period, relate to comparisons between species × P concentration × ± added P cells

P concentration in residue (g kg^{-1})	Incubation period (weeks)									
	0–2		2–4		4–6		6–8		Total (0–8)	
	–P	+P	–P	+P	–P	+P	–P	+P	–P	+P
Stylo										
1.1	18	19	7	8	3	6	3	3	31	36
1.3	22	23	10	13	3	6	3	3	38	45
1.7	23	25	12	14	4	6	4	4	44	49
Cowpea										
1.0	10	12	5	6	5	6	5	5	25	29
1.2	14	15	7	7	5	6	6	6	32	34
2.0	15	15	7	7	6	8	6	6	34	36
LSD (p = 0.005)	0.9		0.9		1.3		0.6		1.5	

in extractable P, over the controls without residues, for the means for the two incubation periods.

Extractable P increased with increasing P concentration in the residues, with a larger increase for the stylo residues than for the cowpea residues. There was also a significant effect of grinding, with larger increases in extractable P where the residue had been ground.

The increase in extractable P will have resulted from both the soluble P in the residues (Figure 1) and any mineralisation of organic P. However the recovery of the P released from the residues by the extractant will be less than complete because of sorption of P by the soil. Increased sorption of P with time is the likely explanation of why extractable P declined when the

Table 4. Increase in extractable P in soil following incubation with ground and unground residues of varying phosphorus concentration, and percentage of added P extracted. Data are averages of units incubated for 4 and 8 weeks. LSD relates to comparisons between species × P concentration × effect of grinding cells

P concentration in residue (g kg^{-1})	Extractable P (μg g^{-1})		as % of added P	
	Ground	Unground	Ground	Unground
Stylo				
1.5	31	25	41	33
2.4	38	33	32	28
3.6	40	36	22	20
5.1	50	45	20	18
Cowpea				
1.3	15	14	23	22
2.8	22	20	16	14
4.0	33	28	16	14
5.1	45	42	18	17
LSD (p = 0.05)	2.1			

incubation period was increased from 4 to 8 weeks. When the increase in extractable P is expressed as a proportion of the P added in residues (Table 4), the apparent recovery decreases with increasing P concentration in the residues. The recovery of added P was higher for the stylo residues than for cowpea, which is consistent with the N mineralisation data (Table 2), though the proportion of added P that was extracted was less than the proportion of added N that was mineralised.

Implications for cycling of nitrogen in crop residues

N mineralisation was affected by the P concentration in the residue and we infer that the reduction in mineralisation for residues with low P concentration is a direct P effect. It is possible that plants grown under P limited conditions might have different composition from plants grown with adequate P, but within a suite of samples grown in either the field or the glasshouse there was little change in the quality factors that are known to influence the rate of mineralisation, for example C:N ratio, lignin and polyphenol concentrations.

The use of leguminous plants to fix N and contribute to the overall N balance of farming systems is widely recognised, for example as green manure crops, in rotations, or in intercropping systems. Where growth of the legumes is restricted by an inadequate P supply, N fixation is also reduced (Nguluu, 1993). The results of this work suggest that a second effect can arise where the legumes are grown under P stress in as much as the rate of mineralisation of crop residues will be reduced due to low P concentration in the residues. Although the magnitude of the effect was not large, it will have an influence on the synchrony of N supply from the residues and demand by a crop, a consideration that is important when endeavouring to maximise the utilisation of residue N by the crop (Myers et al., 1994).

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