Chapter (non-refereed)

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Mineralization of organic phosphorus in relation to soil factors, determined using isotopic ^{32}P labelling

A F HARRISON
ITE, Merlewood Research Station, Grange-over-Sands

1 Introduction

The mineralization of organically bound phosphorus, which forms a major fraction of the soil P capital (Harrison 1979), is essential to the maintenance of the phosphorus cycle and the replenishment of the available P in the soil in forest ecosystems (Harrison 1985). The trees and ground flora, in a local phosphorus-deficient deciduous woodland — Meathop Wood — take up about 11.4 kg P ha^{-1} yr^{-1} from the soil, and this amount is about the average annual uptake for temperate woodlands and forests. If it is assumed that all the P taken up by the vegetation is derived from mineralization of organic phosphorus in the top 5 cm of the soil profile, then the average rate of mineralization required through the year is only about 95 mg P m^{-2} $month^{-1}$ or 4 μg P g^{-1} soil $month^{-1}$. This amounts to about 0.2% to 1% of the organic phosphorus pool in Lake District woodland soils each month. Furthermore, small differences in mineralization rates at the level of <5 μg P g^{-1} soil $month^{-1}$, caused by variation in soil conditions or site management factors, could be quite important in influencing the productivity of both the local woodlands and other UK forest plantations, as they are frequently P-deficient. These rates of mineralization are extremely low.

These conclusions are probably relevant to most ecosystems, for only a small proportion of the soil organic phosphorus needs to be mineralized to provide significant quantities of inorganic phosphorus to plants (Greb & Olsen 1967). With such low rates of mineralization being potentially important, how do we attempt to determine the factors, such as soil and environmental conditions and site management, which have a controlling influence on the rate of soil organic phosphorus mineralization?

At ITE's Merlewood Research Station, we have tried to determine organic P mineralization rates using the conventional incubation methods, but the results from such techniques are far from satisfactory for local woodland soils. Often reductions in the labile organic phosphorus, that extractable in Olsen's bicarbonate (Bowman & Cole 1978a), during incubation are not paralleled by an apparent and equivalent increase in inorganic phosphorus, so we cannot be confident that mineralization is actually taking place. Only the net changes in the labile organic and inorganic phosphorus are being measured by these techniques, and both may change in their extractability during incubation. Also, error terms on the measurements are large, particularly in relation to the low rates of the mineralization required in the ecosystem (see Halstead *et al.* 1963), so that few statistically significant changes in organic or inorganic phosphorus contents of soils are detected on incubation, even after incubation for a period of 6 months (Harrison 1982a). The conventional techniques of incubation appear to be too insensitive and imprecise to answer the problem of how soil and environmental conditions and site management factors influence organic phosphorus mineralization rates in woodland and forest soils.

Consequently, there is a need for another approach to the assay of comparative rates of organic phosphorus mineralization in these woodland soils. I have developed a method in which ^{32}P-labelled ribose-nucleic acid (RNA is a component of the labile fraction of soil organic phosphorus — Bowman & Cole 1978b) was added to soils, which were subsequently incubated. The amount of mineralization was determined following the recovery of the ^{32}P from soils and its partitioning into organic and inorganic forms. The main attribute of the method is the considerably improved sensitivity due to the use of ^{32}P as a tracer and its capacity to provide gross, rather than net, conversion rates. It was sensitive enough to detect highly significant differences in rates of mineralization in the woodland soils, after only 24 h incubation at 13°C, the period being shorter and the temperature being lower than in commonly used incubation methods.

2 Soils and methods

Soil samples were collected from the 0—5 cm depth (after the removal of the litter layer) from brown earth, brown podzolic and rendzina profiles from 13 different Lake District woodlands. Twenty-five samples (1—3 per woodland) were collected in October 1973, and a similar set was obtained the following May. Soils were sieved (2 mm) to remove stones and root material, but were otherwise used in a fresh condition. They varied considerably in their physical and chemical properties (Harrison 1982b).

Subsamples of each fresh soil were compacted into polystyrene 'sterilin' tubes, so that each tube contained 10 cm^3 soil at its natural bulk density. After equilibrating the soils at 13°C (the average soil temperature at the times of the year sampled)

for one day, 1 ml of ^{32}P-RNA (containing 97 μg RNA ≡ 7.8 μg P, with average molecular weight 30 000 and a specific activity of *c* 130 GBq per 10^{-3} M) was distributed in the soil of each tube, using a hypodermic syringe. The ^{32}P-RNA was added in an amount less than generally found to be present in soils. The tubes were incubated for 24 h at 13°C, after which the soil in each tube was mixed and divided into 4 equal weighed subsamples, each of which was added to 100 ml of an extractant solution in a polypropylene bottle. The suspension was then subjected to ultrasonic vibration (50 W, 20 kHz at 6—8 μ amplitude) for 5 min. Bottles were shaken subsequently overnight on a reciprocal shaker in a waterbath at 18°C. Subsamples of the suspension were centrifuged and the ^{32}P-labelled phosphorus in the supernatant partitioned into inorganic and organic forms, using a method similar to that of Martin and Doty (1949); inorganic ^{32}P transferred into the isobutanol-benzene layer with an efficiency of >99.5%. Total ^{32}P recovered in the original supernatant and inorganic ^{32}P recovered in the organic layer were determined, after addition to fluor, by liquid scintillation spectrometry. To determine the degree of ^{32}P-RNA hydrolysis caused by the extraction and separation procedures, control samples were also prepared for all soils; ^{32}P-RNA was added to soil, immediately extracted, and partitioned into organic and inorganic forms. Recoveries of ^{32}P from the 50 soils ranged from 56±2 to 104±6%, with only 10 soils having recoveries of <80%; recovery was negatively related to the amounts of extractable calcium (r^2=0.71**) and clay (r^2=0.42**) in the soils. In the calculations of the mineralization rate, it has been assumed that ^{32}P not recovered from the soil was apportioned between organic and inorganic forms in the same ratio as that extracted. Further details of the methodology are presented in Harrison (1982a).

3 Results

The rates of net mineralization of the ^{32}P-RNA in the 50 soils ranged from −29 to 190 ng P cm^{-3} soil day^{-1}, with a least significant difference of about 10 ng. These rates of mineralization are equivalent to 0.75 to 5.7 μg P cm^{-3} soil month^{-1}. The negative values are attributable to net immobilization of the small amount of inorganic ^{32}P present in the ^{32}P-RNA preparation. Net immobilization of inorganic P occurred in 12 of the October samples, with no May samples having negative mineralization rates. Partially as a result, soils collected in October had lower rates of mineralization (9.2±7.8 ng P cm^{-3} soil day^{-1}) than those collected in May (45.9±9.8 ng P cm^{-3} soil day^{-1}). Soils over limestone, irrespective of the time of the year collected, had higher rates of mineralization (56±14.2 ng P cm^{-3} soil day^{-1}) than those soils over slates, shales and andesite-tuff rocks (10±4.2 ng P cm^{-3} soil day^{-1}). There were also strong interactive effects of the time of year and the underlying geology, as shown by covariance analysis, on the relationships between mineralization rate and soil chemical and physical properties (Table 1). In general, however, strong and significant positive relationships were found with soil pH and extractable calcium contents, and available phosphorus contents (expressed as a combined function of water-soluble, isotopically exchangeable and Olsen's bicarbonate extractable inorganic phosphorus) across all soils (Table 2). Fifty per cent of the variation in ^{32}P-RNA mineralization rate was accounted for by the 3 'available' P variables, sometimes more when the soils were partitioned according to the geology and time factors. A significant negative relationship was also found between mineralization rate and the Olsen's bicarbonate extractable organic phosphorus content (particularly when expressed as a percentage of the total organic phosphorus) of soils; the latter is considered to be the labile fraction of soil organic phosphorus (Bowman & Cole 1978a). Relationships with phosphatase activity and respiration rate in the soils were found only for soils overlying slates, shales and andesite-tuff rocks. Around 90% of the variation in rates of mineralization in the soils was accounted for by significant soil properties (not all the measured soil properties are mentioned in this paper). Further details of the relationships between the rate of ^{32}P-RNA mineralization and soil properties are presented in Harrison (1982b).

4 Discussion

The question of whether or not analytical procedures to be applied to soils are sensitive

Table 1. Effects[1] of geology and time of sampling on relationships between ^{32}P-RNA mineralization rate and properties in 50 soils

Property	Geology[2] Slope	Geology[2] Elevation	Time[3] Slope	Time[3] Elevation
pH	NS	NS	*	***
Extractable Ca	NS	NS	**	*
^{32}P-exchangeable P_i	NS	NS	NS	NS
Water-soluble P_i	NS	***	**	**
Extractable P_i	*	***	NS	*
Extractable P_o	*	NS	NS	**
Extractable P_o % P_t	NS	NS	*	**
Phosphatase activity	*	***	NS	**
Soil respiration	NS	**	NS	*

[1] As shown by covariance analysis
*, **, *** Significant at P<0.05, P<0.01 and P<0.001 respectively
[2] Group 1 Soils on slates, shales and Andesite—tuffs
Group 2 Soils on limestone
[3] Group 1 October sampling
Group 2 May sampling

Table 2. Relationships between ^{32}P-RNA mineralization rates and some soil properties[1]

Property	All soils		Geology: Slates & Andesites		Geology: Limestone		Time: October		Time: May	
	±	r^2	±	r^2	±	r^2	±	r^2	±	r^2
pH	+	0.42**	(+)	0.48**	+	0.24*	+	0.30**	+	0.67**
Extractable Ca	(+)	0.49**	+	0.62**	+	0.27*	(+)	0.44**	+	0.73**
^{32}P-exchangeable P_i	+	0.42**	+	0.35**	+	0.27*	(+)	0.56**	+	0.47**
Water-soluble P_i	+	0.14**	+	0.24**	(+)	0.36*	+	0.77*	NS	
Extractable P_i	+	0.27**	+	0.24**	+	0.28*	+	0.79**	NS	
Extractable P_o	(−)	0.24**	NS		NS		NS		(−)	0.42**
Extractable P_o % P_t	(−)	0.43**	(−)	0.32**	−	0.24*	−	0.27**	(−)	0.52**
Phosphatase activity	NS		(+)	0.60**	NS		NS		NS	
Soil respiration	+	0.10*	(+)	0.51**	NS		NS		NS	

[1] Properties expressed cm^{-3}; ( ) significant quadratic term

enough to measure the likely biologically significant differences in the parameters of particular interest in a study should always be asked, preferably before embarking on the study. In the case of the present method, the use of ^{32}P as a tracer has markedly increased the sensitivity obtainable and also made it possible to measure both mineralization and microbial immobilization processes at the same time. The mineralization rates measured, using this method, are of the same order as those likely to be significant in the replenishment of available phosphorus in the local woodland soils. The method also has the following advantages: (i) it generates reasonably precise estimates of mineralization in soils; (ii) the pattern of the relationships between mineralization rate and soil properties appears to be theoretically acceptable; and (iii) it is sensitive enough to be able to detect interactive effects of geology and season in those relationships as well. Moreover, a very high proportion of the variation in the ^{32}P-RNA mineralization rates between soils could be accounted for by the measured soil properties.

As a consequence, it appears to be quite suitable for studies determining the various influences of soil conditions, crop type, environmental and land management factors on rates of organic phosphorus mineralization in soils. It could also provide answers to questions about the relative uptake by plants and micro-organisms of phosphorus from the labile organic phosphorus pool during the growing season (Tate 1984). Analysis of the effects of all these factors, through the development of predictive models, is important, as the mineralization of organic phosphorus is a key process in the phosphorus cycle of managed or 'natural' terrestrial ecosystems and the maintenance of soil fertility (Harrison 1985, 1987).

5 Summary

An isotopic method, to examine the mineralization of labile organic phosphorus in soils at the low rates likely to be significant in the nutrition of forest trees, is briefly outlined. The method uses ^{32}P-labelled RNA, added at 780 ng P cm^{-3}, and allows adjustments to be made for its hydrolysis induced during the soil extraction and organic-inorganic separation phases of the procedure. Rates of mineralization in 50 soils, with pH ranging from 3.1 to 7.5, varied from −29 to 190 ng P cm^{-3} when incubated for 24 h at 13°C. The rates of mineralization were primarily related to soil pH and extractable calcium, and nearly 50% of their variation was related to the availability of P in the soils. Using covariance analysis, interactions of site geology and time of the year were found in the relationships between mineralization rate and a range of soil properties. Over 90% of the total variation in rates of mineralization could be accounted for by soil chemical, physical and biological properties. The method is considered to be useful in understanding the influences of soil conditions and environmental factors on the rate of organic phosphorus mineralization in soils.

6 References

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