FACTORS CONTROLLING THE RELEASE OF PHOSPHORUS FROM DECOMPOSING WHEAT ROOTS

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Abstract

An increase in incubation temperature from 5 to 45°C significantly ($P<0{\cdot}001$) increased the release of radioactivity into water solution from intact root systems severed from wheat plants labelled with ^{32}P. Sterile roots incubated at 45°C released >90% of total radioactivity into solution, mainly as orthophosphate, showing an extensive dephosphorylation of organic phosphorus components by plant enzymes. Factors favouring microbial growth around the dead roots caused a slight reduction in ^{32}P activity in solution. Air-drying at 20–40°C prior to incubation resulted in a rapid release of ^{32}P activity from the roots which was independent of the incubation temperature.

It is concluded that root phosphorus can be released directly into soil solution, mainly as orthophosphate, as the result of autolysis and does not necessarily become incorporated into the organisms decomposing the roots.

The amounts of phosphorus contained in different components of the total soil biomass were estimated and the conditions favouring release of this phosphorus into soil solution are discussed.

I. INTRODUCTION

An earlier study (Martin 1970) demonstrated that a significant proportion of the organic phosphorus pool present in soil microflora was released into solution from dead or senescent cells with the phosphate ester bond intact. It was suggested that there would be a similar release of phosphate esters from decomposing roots and other plant debris. Organic phosphorus from either or both of these sources could contribute to the pool of soil organic phosphorus and could also represent a source of phosphorus for new plant growth.

Previous investigations of the transformations of phosphorus occurring during the decomposition of plant material (Chang 1939; Kaila 1949; Birch 1961; Jones and Bromfield 1969) showed an extensive microbial synthesis of organic phosphorus from orthophosphate. The subsequent release of this phosphorus as orthophosphate was very slow and Birch (1961) suggested that little of the original plant organic phosphorus was mineralized during a decomposition period of 3 months. However, in all of these studies the plant material was present as a solid mass of chopped or ground air-dried leaves, stems, or roots and was not distributed through soil. It seemed possible that the marked immobilization of the plant phosphorus resulting from its incorporation

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into microbial cells was largely a consequence of the experimental conditions, since microbial populations in decomposing green manure may be 10 times higher than the normal population on root surfaces (Clark 1949). It seemed desirable to determine the degree to which the inorganic and organic phosphorus components contained in plant roots are released into solution with decomposition conditions simulating those existing in undisturbed arable and pasture soils.

In the present study, intact root systems from wheat seedlings labelled with ^{32}P were buried in soil columns and a systematic study made of the factors determining the proportion of the initial radioactivity which was recovered in water solution leached from the columns. The decomposition of ^{32}P-labelled roots from mature wheat plants was studied in a soil-free system, to avoid sorption by soil particles of radioactivity leached from the roots.

II. Materials and Methods

(*a*) *Plant Culture*

Wheat seedlings, surface-sterilized with calcium hypochlorite, were grown in nutrient solution (two seedlings per tube containing 30 ml solution) using the procedures described by Ridge and Rovira (1971) except that KH_2PO_4 was omitted from the medium. The seedlings were illuminated for 16 hr/day by mercury vapour lamps providing 21,000 lux at plant level, with light and dark temperatures of 21 and 15°C respectively. Carrier-free [^{32}P]orthophosphate (A.A.E.C. P2B1) was added to the cultures (5 μCi) as a sterile solution 7 days after seed germination and the plants were normally harvested after 10 days growth. Unlabelled roots were obtained from plants grown identically but without addition of [^{32}P]orthophosphate. Sterility checks were made at harvest and plants from contaminated tubes were rejected.

Wheat plants of increasing maturity were grown in Mt. Compass sand (coded Uc2·3, Northcote 1965), following the procedures described by Martin (1971). Pots, each containing five plants, were harvested approximately 2, 6, and 10 weeks from seeding, with plant development corresponding to a Feekes scale (Large 1954) of 2 (tillering), 10 (heading), and 10.5.1 (flowering) respectively. Carrier-free [^{32}P]orthophosphate was added to all pots 7 days after seeding, at levels of 25 and 50 μCi for the pots harvested at 2 or 6 weeks, and 10 weeks respectively. This provided levels of radioactivity in the plant material at each harvest comparable to that obtained with the seedlings grown in nutrient solution. Measurements of radioactivity in the soil after harvest showed more than 90% uptake of the [^{32}P]orthophosphate by the plants.

(*b*) *Root Decomposition Conditions*

(i) *Soil Columns*

The seedlings were removed from the nutrient solution and the roots held in running tap water for 15 min. The plants were lightly blotted and the tops were separated from the roots at the junction with the seed. Intact root systems from individual plants were placed without compression in 15-ml glass centrifuge tubes and sufficient soil poured into the tube to completely cover the roots (10 g of Mt. Compass sand). The soil was immediately wet with distilled water to a point just under saturation and the tube capped and incubated as required for the varying treatments. In some experiments Mt. Compass sand was replaced by glass beads (Ballotini number 12).

(ii) *Soil-free System*

The soil was washed away from the plant roots with running tap water and the roots thoroughly rinsed with distilled water. Tops were removed, and the intact root system from individual plants was placed in 250-ml Erlenmeyer flasks containing 50 ml of distilled water. The flasks were covered with polythene film and incubated for 3 days at the required temperature. In some treatments, the roots were air-dried on trays in incubators without forced circulation, prior to incubation.

(c) *Leaching Procedures*

Soil columns were leached with distilled water, the solution being drained through a hole pierced in the bottom of the tube with a hot wire. A small plug of glass wool retained the soil. The volume of water used and the period between leachings varied and the values are given in individual experiments. In some experiments a solution containing 0·05% glucose+0·01% asparagine was used for wetting and leaching the tubes. Tubes were capped between leachings to prevent water loss.

Soil grown roots held in Erlenmeyer flasks were leached once only after the 3-day incubation. The free water was decanted from the flasks and the roots rinsed with two lots of 25 ml of water. The total volume of leachate was adjusted to 100 ml.

(d) *Measurement of Radioactivity*

The amount of ^{32}P activity present in leachates and in extracts and digests of roots was measured by planchet counting, with correction for background and decay. Leachates from soil columns were centrifuged to remove clay particles, prior to counting.

The initial activity present in the seedling roots was measured before burial in the soil columns by immersing the intact root system in distilled water in a scintillation vial and counting Cerenkov radiation with a liquid scintillation counter. The ^{32}P activity contained in the roots was separated into acid-soluble and alkaline-soluble fractions by procedures described previously (Martin 1973). The total ^{32}P activity in the roots measured by Cerenkov counting was related by a constant factor to the total ^{32}P activity measured by planchet counting of the root extracts and residue.

The initial radioactivity present in the soil-grown plants was taken as the sum of the ^{32}P activity present in the leachate and in the residual root. The residual value was measured on a nitric+perchloric acid digest of the leached roots.

III. Statistical Methods

(a) *Experimental Design*

The seedling root experiments were designed as complete factorial experiments with at least three replicates of each treatment. The number of treatments was restricted by the time required for measurement of ^{32}P activity in the leachates.

Balanced incomplete block designs were used for the soil-grown plants to minimize pot differences. The designs and factors considered for each experiment are listed below.

Experiment A: 4×2 factorial with six replicates. The factors were incubation temperature (10, 25, 40, 55°C)×root age (14, 21 days). Tubes were leached with 10 ml of water at intervals of 3–4 days for a total of five leachings.

Experiment B: 2×2×4 factorial with three replicates. The factors were column packing (Mt. Compass sand, glass beads)×incubation temperature (10, 40°C)×^{32}P source (^{32}P-labelled roots, [^{32}P]orthophosphate solution, [^{32}P]orthophosphate solution+unlabelled roots, [^{32}P]orthophosphate solution+glucose–asparagine solution). Tubes were leached with 25 ml of water or glucose–asparagine solution for a total of six leachings—Nos. 1–4 at 24-hr intervals and Nos. 5 and 6 at 72-hr intervals.

Experiment C: 6×2×2 factorial with six replicates. The factors were incubation temperature (5, 15, 25, 35, 45, 55°C)×^{32}P source (^{32}P-labelled roots, [^{32}P]orthophosphate solution+unlabelled roots)×leaching solution (distilled water, glucose–asparagine solution). Tubes were leached with 25 ml of liquid for a total of six leachings—Nos. 1–4 at 24-hr intervals and Nos. 5 and 6 at 48-hr intervals.

Experiment D: Balanced incomplete block—11 pots, 11 treatments, five replicates, five plants per pot. Sets of plants were harvested 2, 6, and 11 weeks from seeding. The factors were incubation temperature (5, 15, 25, 35, 45°C)×preincubation treatment (fresh roots, roots air-dried at 55°C).

Experiment E: Balanced incomplete block—11 pots, 11 treatments, five replicates, five plants per pot. Plants were harvested 2 weeks from seeding. The treatments consisted of a factorial set of three drying temperatures (20, 30, 40°C) × three times (4, 7, 14 days) plus two treatments in which roots, kept moist by a wrapping of polythene film, were held at 20°C for 4 or 14 days. All roots were then incubated in 50 ml of distilled water at 5°C for 3 days.

Experiment F: Balanced incomplete block—11 pots, 11 treatments, five replicates, five plants per pot. Plants were harvested 9 weeks from seeding. The factors are shown in Table 5.

(*b*) *Calculation of Variables*

Initially, the only variable studied was the total amount of ^{32}P activity recovered over all the leachings, expressed as a percentage of the initial amount. Most of the treatment means were in the range 10–90% so that variances were constant and transformation was unnecessary. Where some of the data were in the extreme ranges, estimates of variance were based on the observations in the 10–90% range.

Subsequently, it was recognized that with different treatments there could be a large difference in the proportion of the ^{32}P activity recovered in the first leaching which was not reflected in the total recovery. A method proposed by Rao (1958) provided a simple and exact estimate of average decay rates.

This procedure, by a suitable choice of time metameter, approximately linearizes each decay curve allowing calculation of rates. In brief, if $y_1 \ldots\ldots, y_p$ represent the amount of ^{32}P released at each leaching and g_i represents the length of the interval between the $i-1$th and the ith time points on the transformed axis g_i, then the estimated rate is given by

$$b = \Sigma\, y_i g_i / \Sigma\, g_i^2.$$

As no *a priori* estimates of g_i were available, a suitable estimate of g_i was taken to be the grand mean of the y_i's (Rao 1958). The data were transformed by $\log_e$ in an attempt to obtain as near as possible a linear trend with respect to the time metameter.

The method has the advantage of transforming the time scale according to the amount of ^{32}P released at each leaching. Consequently the scaling takes account of the fact that, irrespective of the incubation time, a large proportion of the ^{32}P was released at the first leaching. It was for this reason that polynomial forms were found inappropriate.

Covariance analysis was used to adjust the rate coefficients for the differing initial amounts of ^{32}P present in the system. The values in the tables are the absolute mean rates for each treatment together with the estimated standard errors. A large rate value indicates a rapid release of ^{32}P into the leachates.

IV. Results

(*a*) *Validation of Techniques*

(i) *Growth of ^{32}P-labelled Plants*

It was desirable to have a high proportion of the ^{32}P label incorporated into organic phosphorus pools, particularly acid-insoluble components. Otherwise, ^{32}P release could be simply leakage of [^{32}P]orthophosphate, without any extensive degradation of cell structure.

Table 1 shows the distribution of ^{32}P activity in the acid-soluble fraction from roots with different growth conditions. It was apparent that plants grown in nutrient solution without orthophosphate, with either pulse or continuous labelling, provided suitable material. A separate experiment established that when plants were pulse-labelled there was a direct relationship between the ^{32}P activity in the roots and in the nutrient solution, as shown in the following tabulation:

^{32}P activity in nutrient solution (μCi)	5	10	20
^{32}P activity in intact roots (counts/min)	$0{\cdot}47\times10^6$	$1{\cdot}03\times10^6$	$1{\cdot}78\times10^6$

Thus it was possible to control both the total radioactivity present in the excised roots and the relative distribution of ^{32}P between the acid- and alkaline-soluble fractions.

TABLE 1

EFFECT OF GROWTH CONDITIONS ON THE DISTRIBUTION OF ^{32}P ACTIVITY IN ACID-SOLUBLE FRACTIONS OF WHEAT ROOTS

^{32}P application	^{32}P level in nutrient solution (μCi/30 ml)	^{31}P level in nutrient solution (μg P/ml)	^{32}P activity in acid-soluble fraction (% of total)
Continuous	2	Nil	54·8
		31	79·0
Pulse	20	Nil	56·6
		8	77·5
		31	81·3
S.E.			2·20

(ii) *Recovery of ^{32}P Activity from Soil Columns*

The experimental design required that the ^{32}P activity leached from soil columns containing decomposing wheat roots should reflect the amount of ^{32}P released from the roots. Hence there should be a minimum sorption of radioactivity by the soil. It was also necessary that biological properties of the soil, such as the microbial population and organic matter content, which could influence root decomposition should be comparable to those of arable soils.

TABLE 2

^{32}P ACTIVITY RECOVERED IN LEACHATES FROM SOIL COLUMNS

Columns (six replicates) containing 10 g soil plus 0·25 μCi carrier-free [^{32}P]orthophosphate were saturated with distilled water and held at 25°C. Columns were leached with 10 ml distilled water after 3, 7, 11, 15, and 19 days incubation

Soil	Average release rate*	Percentage recovery†	Soil	Average release rate*	Percentage recovery†
Mt. Compass	2·48	70·9	Marion Bay	0·03	2·0
Mt. Burr	2·37	67·9	Nangkita	0·20	8·2
Young	2·05	63·6	Moon Hills	0·79	35·2
Foul Bay	0·02	1·1			

* Defined in Section III(*b*). S.E. = 0·068.

† Sum of the ^{32}P activity in the five leachings expressed as a percentage of the ^{32}P activity added to the soil. S.E. = 1·31.

Table 2 presents the rate of release and the percentage recovery in water leachates of [^{32}P]orthophosphate added to columns of sandy soils collected in South Australia.

It was evident that Mt. Compass sand which had been collected from a depth of 0–20 cm from a permanent pasture, air-dried, and sieved through a 1-mm mesh screen, would be suitable.

Additional experiments showed that there was no significant difference in the recovery in water leachates of [^{32}P]orthophosphate added to 10 g of Mt. Compass sand with leachate volumes of 25, 50, 75, or 100 ml. The period of time between addition of [^{32}P]orthophosphate and leaching had no significant effect on the recovery of ^{32}P activity up to 72 hr but there was a small ($P<0\cdot05$) decrease in recovery at 96 hr.

(*b*) *Release of ^{32}P from Labelled Roots*

(i) *Seedling Roots*

A preliminary experiment showed that the release of ^{32}P activity from labelled roots was strongly temperature-dependent. Six leachings of soil columns containing ^{32}P-labelled roots held at 4 and 25°C gave values for total percentage recovery of ^{32}P activity of 17·9 and 70·3 respectively (S.E. 1·95).

Other factors which were considered likely to influence the release of phosphorus from the roots were age and desiccation of the roots and the level of microbial activity. Multifactorial experiments were designed to study the individual effect of each factor and their interactions.

Experiment A, which tested the effect of the age of the buried roots and the temperature at which they were decomposed, showed no significant difference in the rate of release or total recovery of ^{32}P activity between root ages of 14 and 21 days for any of the temperatures studied. There was a significant ($P<0\cdot001$) increase in the release of ^{32}P with increasing temperatures, with an apparent levelling in the rate of release above 40°C, as shown in the following tabulation:

Temperature (°C)	10	25	40	55	
Average release rate	0·73	0·97	1·15	1·15	S.E. 0·062
Recovery of ^{32}P in leachates (%)	63·0	71·4	79·8	81·1	S.E. 2·82

Experiment B was designed to test if the values for the percentage recovery and average release rate of ^{32}P activity from decomposing roots were affected by retention on the soil or by incorporation into microbial cells. The results are given in Table 3 and show that there was a limitation by the soil on the rate of release of radioactivity from labelled roots. There was no significant difference between the release rates from Mt. Compass sand and glass beads at 10°C but there was a significantly ($P<0\cdot001$) higher rate of release from glass beads than soil at 40°C. The release of ^{32}P activity was also higher ($P<0\cdot001$) from columns of glass beads than soil columns, for each of the treatments with [^{32}P]orthophosphate.

The addition of unlabelled roots had no effect on the release of [^{32}P]orthophosphate activity from either soil or glass beads but the use of a dilute solution containing glucose and asparagine for leaching the columns caused a significant ($P<0\cdot001$) reduction in the percentage recovery of [^{32}P]orthophosphate activity from the soil columns.

The contrasting effects of glucose–asparagine solution and unlabelled roots on the release of ^{32}P was studied over a wider range of temperatures (5, 15, 25, 35, 45, 55°C) in experiment C. Columns of glass beads containing ^{32}P-labelled roots or

unlabelled roots+[^{32}P]orthophosphate solution were wet and leached with either distilled water or glucose–asparagine solution, using the procedures described for

TABLE 3

RELEASE OF ^{32}P ACTIVITY FROM ^{32}P-LABELLED ROOTS OR [^{32}P]ORTHOPHOSPHATE SOLUTION ADDED TO COLUMNS OF SOIL OR GLASS BEADS

^{32}P source†	Average release rate: Soil		Average release rate: Glass beads		Total ^{32}P activity in leachates* (% of initial root activity): Soil		Total ^{32}P activity in leachates* (% of initial root activity): Glass beads	
	10°C	40°C	10°C	40°C	10°C	40°C	10°C	40°C
Labelled roots	0·39	0·80	0·29	1·26	73·6	89·3	50·9	91·2
Orthophosphate	0·68	0·51	1·73	1·56	79·8	78·1	95·6	97·3
Orthophosphate+ unlabelled roots	0·63	0·53	1·62	1·68	79·4	77·8	94·2	96·0
Orthophosphate +glucose–asparagine‡	0·53	0·44	1·72	1·63	66·6	69·0	95·0	95·5
S.E.	0·066				2·01			

* Sum of the ^{32}P activity recovered in six leachings, each with 25 ml liquid—Nos. 1–4 at 24-hr intervals, Nos. 5 and 6 at 72-hr intervals.

† Added to 10 g Mt. Compass sand or size 12 Ballotini glass beads.
Labelled roots: mean ^{32}P activity—333,500 counts/min; mean weight (fresh) —45·8 mg.
Unlabelled roots: mean weight (fresh) —46·3 mg.
Orthophosphate: 0·5 ml carrier-free [^{32}P]orthophosphate solution containing 300,900 counts/min.

‡ Glucose–asparagine: columns saturated and leached with a solution containing 0·05% glucose+0·01% asparagine.

experiment B (Table 3). The means for individual treatments showed no clearly defined set of conditions giving the maximum difference between the two leaching solutions, but the overall means were significantly ($P<0·001$) different as shown in the following tabulation:

Leaching solution	Water	Glucose–asparagine	S.E.
Average release rate	1·129	0·885	0·030
^{32}P recovery in leachates (%)	71·5	61·3	1·032

Increasing incubation temperatures from 5 to 55°C significantly ($P<0·001$) increased the rate of release of radioactivity from ^{32}P-labelled roots, reaching a maximum in the range 35–45°C in confirmation of the previous experiment. There was no effect at temperatures <45°C on the release of ^{32}P activity from unlabelled roots+[^{32}P]orthophosphate.

The release of ^{32}P from sterile roots was studied by transferring intact root systems directly from growth tubes into scintillation vials containing 15 ml of sterile distilled water. The vials were incubated for 96 hr at temperatures of 5, 25, and 45°C (12 replicates). ^{32}P activity released into solution was estimated as the difference of the corrected Cerenkov radiation measured with the roots suspended in water and after their removal from the vials. Sterility checks, made when the roots were

removed, showed 10, 4, and 12 sterile vials from the 5, 25, and 45°C sets respectively. Sterile solutions from each set were pooled and fractionated by anion-exchange chromatography and one-dimensional thin-layer chromatography (Martin 1970). The percentage of the total ^{32}P activity recovered in sterile solution at the three incubation temperatures and the percentage of the extracted ^{32}P activity present in the orthophosphate fraction from the resin columns are shown in the following tabulation (S.E. in parenthesis):

Incubation temperature (°C)	5	25	45
Percentage ^{32}P recovery in solution	25·2 (1·6)	47·8 (6·3)	95·5 (1·0)
Percentage of extracted ^{32}P as orthophosphate	91·2	93·7	94·8

Radioautographs of the developed chromatograms, which had received a comparable loading of ^{32}P activity, showed for each temperature 6–8 areas of weak radioactivity separated from the intense orthophosphate spot.

(ii) *Roots from Soil-grown Plants*

A single measurement of ^{32}P release into water solution from roots held in Erlenmeyer flasks was employed for the studies with soil-grown plants. Experiments with ^{32}P-labelled roots held in water at 5, 25, and 45°C for periods from 4 to 72 hr showed an increase in ^{32}P activity in solution up to 48 hr at each temperature but after this period the values appeared to stabilize.

TABLE 4

EFFECT OF INCUBATION TEMPERATURE AND AIR-DRYING PRIOR TO DECOMPOSITION ON THE RELEASE OF RADIOACTIVITY FROM ^{32}P-LABELLED ROOTS FROM PLANTS OF INCREASING AGE

Results are percentage of initial ^{32}P activity recovered in solution*

Temp. (°C)	Plants 2 weeks old†		Plants 6 weeks old		Plants 11 weeks old	
	Fresh‡	Air-dry‡	Fresh	Air-dry	Fresh	Air-dry
5	14·4	83·9	4·5	78·7	6·4	79·0
15	37·2	83·4	16·2	72·6	19·7	74·9
25	66·2	87·6	32·9	81·0	24·8	75·2
35	85·1	88·2	59·8	85·6	60·0	82·1
45	93·6	93·2	83·3	89·8	83·2	86·5
S.E.	1·18		2·47		2·10	

* Initial ^{32}P activity = sum of ^{32}P activity in solution (50 ml incubation fluid + 50 ml rinse fluid) + residual activity in incubated roots measured after digestion with nitric–perchloric acids.

† Mean weight of roots (dry): 2 weeks, 50·2 mg; 6 weeks, 139·5 mg; 11 weeks, 286·4 mg. Mean phosphorus content (% dry weight): 2 weeks, 0·55; 6 weeks, 0·087; 11 weeks, 0·094.

‡ Fresh: roots transferred to incubation flasks immediately after harvest; air-dry: roots held at 55°C on open trays for 4 days before transfer to incubation flasks.

The effect of incubation temperature, plant age, and desiccation of the roots prior to decomposition was studied in experiment D. The results are presented in Table 4 and show a significant ($P<0·001$) increase in the release of ^{32}P activity from

fresh roots with increasing temperature for all plant ages. However, when the roots had been air-dried at 55°C prior to the decomposition cycle, the temperature effect virtually disappeared. There was a uniformly high release of ^{32}P activity which was significantly ($P<0\cdot001$) higher than from fresh roots except at the highest temperatures: 35°C and 45°C for plants 2 weeks old and 45°C for plants 6 and 11 weeks old.

Experiment E studied in more detail the effect of desiccation of the roots, using drying treatments closer to field conditions than the temperature of 55°C employed in experiment D. The treated roots were decomposed at 5°C, to avoid masking the effects of air-drying.

There was a significant ($P<0\cdot001$) difference in the release of ^{32}P activity from roots maintained in a moist condition at 20°C for 4 or 14 days (^{32}P recovery in solution 65·3%) in comparison with roots held at the same temperature but allowed to dry (recovery 84·6%). The S.E. (difference) for this significance was 1·52. There was no difference between the release of ^{32}P activity from roots dried at 20, 30, or 40°C for 4, 7, or 14 days prior to the decomposition treatment.

TABLE 5

RELEASE OF RADIOACTIVITY FROM ^{32}P-LABELLED ROOTS WITH VARYING DESICCATION AND DECOMPOSITION TREATMENTS

Treatment	Desiccation		Decomposition		Initial ^{32}P activity recovered in solution‡ (%)
	Time (days)	Temp. (°C)	Time (days)	Temp. (°C)	
A*	Nil	—	3	5	5·5
B*	Nil	—	3	25	27·3
C*	3	25 (moist)	Nil†	—	21·5
D	3	25	Nil†	—	37·5
E*	4	20 (moist)	3	5	36·4
F	4	20	3	5	46·7
G	2	20	3	5	44·4
H	2	40	3	5	46·3
I	3	20	3	5	51·7
J	3	25	3	5	54·0
K	3	40	3	5	49·0
S.E.					2·00

* Treatments A, B: fresh roots transferred directly to Erlenmeyer flasks containing 50 ml water. Treatments C, E: fresh roots wrapped in polythene film before placement on open trays in incubator, together with remaining treatments.

† "Desiccated" roots were shaken with 50 ml water for 15 min at 15°C, followed by two rinsings each with 25 ml water.

‡ Defined in Table 4.

Experiment F combined several of the desiccation treatments in experiment E with variations in the conditions for decomposition. The results, given in Table 5, confirm in a single experiment the main conclusions from the preceding experiments:

(1) an increased release ($P<0\cdot001$) of ^{32}P into solution from fresh roots with an increase in incubation temperature;

(2) an increased release ($P<0{\cdot}001$) of ^{32}P from roots which have been air-dried prior to the decomposition cycle compared with fresh roots;

(3) the lack of a consistent effect of drying temperature or the time of storage of dried material on the release of ^{32}P from labelled roots.

Table 5 also provides evidence that the increased release of ^{32}P from air-dried roots results largely from changes occurring during the drying period. There was a significant ($P<0{\cdot}001$) increase in the amount of radioactivity immediately extractable by water from roots air-dried at 25°C compared with roots kept moist at the same temperature (treatments C and D).

TABLE 6

TOTAL PHOSPHORUS RELEASED FROM ^{32}P-LABELLED ROOTS WITH VARYING DESICCATION AND DECOMPOSITION TREATMENTS

Treatment*	Initial total phosphorus present in solution (%)	Specific activity (c.p.m./μg P)	
		Water soluble	Residual
A	6·6	1284	1466
D	40·9	1291	1487
F	49·2	1261	1373
J	58·2	1226	1456
S.E.	1·72	84·8	45·1

* As defined in Table 5.

The water leachates and residual roots from several of the treatments listed in Table 5 were also analysed for total phosphorus content. Table 6 shows the values for the percentage of the total phosphorus present in solution for each treatment, together with estimates of the specific activities of the phosphorus fraction in solution and remaining in the roots. There was good agreement, for each treatment, between the percentage recovery in solution of ^{32}P activity and total phosphorus. There was also no difference, significant at $P<0{\cdot}05$, between the specific activities of the phosphorus in solution and the phosphorus retained in the roots, both within and between treatments. Hence it was very unlikely that the ^{32}P activity released from the roots was derived from a particular pool and did not reflect the total phosphorus status in the roots after each of the treatments.

V. DISCUSSION

There was a consistent pattern for the effect of individual factors on the rate of release and percentage release of ^{32}P from labelled wheat roots, despite variation between experiments in the values obtained.

Incubation temperature was the dominant factor controlling the release of ^{32}P activity from roots which had not been air-dried. More than 45% of the radioactivity of fresh roots was acid-insoluble, so that treatments yielding >70% of the initial ^{32}P activity in a water-soluble form must have caused extensive degradation of complex

organic phosphorus components. The experiment with sterile seedling roots showed that the degradation could result solely from the autolytic activity of the plant enzymes.

However, the factor having the greatest effect on the release of phosphorus from wheat roots was desiccation. A very high proportion of the total phosphorus was present in a form readily soluble in cold water after drying for several hours at temperatures of 20–30°C. Barr and Ulrich (1963) have also reported an extensive degradation of organic phosphorus to orthophosphate in plant tissue during air-drying. A comparable degradation of nitrogenous material is indicated from studies by Katznelson *et al.* (1954) who observed the liberation of appreciable amounts of amino acids from plant roots which had been air-dried and re-wet.

There was evidence for an incorporation of ^{32}P activity into microorganisms when a solution of glucose+asparagine was used for wetting the columns and leaching. However, when intact unlabelled wheat roots provided the only nutrient source there was no evidence for the incorporation of [^{32}P]orthophosphate into microbial cells and it appeared that the increase in microbial numbers was not rapid enough to immobilize the ^{32}P before it was leached from the column.

The results of these experiments are in conflict with Birch's (1961) opinion that the mineralization of plant organic phosphorus occurs only slowly. It seems likely that in Birch's experiments re-wetting the finely ground dry plant material promoted a much larger and more rapid growth of microorganisms than occurred with the intact roots in the present experiments.

On the basis of the results presented, it appears that in moist soil at temperatures above 15°C phosphorus can be released from dead roots before an extensive population of microorganisms has built up in response to the fresh organic matter. When a mass of roots has been desiccated, as will occur during summer for many topsoils in southern Australia, the first wetting rain will bring the bulk of the plant phosphorus into solution before any appreciable decomposition of the roots occurs. In both cases, the phosphorus requirements of organisms responsible for the subsequent decomposition of the plant material would have to be obtained in competition with new root growth and the requirements of other soil microflora and microfauna.

A similar situation exists for dead plant material falling onto the surface of soil. Bromfield and Jones (1972) studied the leaching of phosphorus from hayed-off pasture under conditions simulating different rainfalls. Up to 90% of the water-soluble phosphorus, which was mainly orthophosphate and represented 60–83% of the total plant phosphorus, was leached with conditions equivalent to a rainfall of 230 mm in 12 hr, but even low intensity leaching equivalent to 12·5 mm rain removed 27–59% of the water-soluble phosphorus. Wetting the plant material at 20°C without leaching resulted in the microbial formation of water-insoluble phosphorus which commenced 18–30 hr after wetting and was virtually completed between 72 and 192 hr after wetting. The proportion of the phosphorus immobilized varied with the nitrogen and phosphorus status of the plant sample.

An upper limit for the amount of phosphorus which could be released from plant roots in a desiccated soil can be estimated from the values given by Troughton (1957) for the weight of roots produced by herbage grasses. Assuming a phosphorus content of 0·1% of dry weight, the calculated values range from 4 to 8 p.p.m. phosphorus. A much larger pool of phosphorus is contained in the biomass of soil

microflora and micro- and mesofauna. Estimates of the dry weight of the biomass of microflora calculated from direct soil counts range from 40–400 p.p.m. (Alexander 1961) to 300–3000 p.p.m. (Clark 1949). The biomass of soil animals is equally uncertain but a value of 1000–1500 p.p.m. dry weight given by Russell (1961) is probably a conservative estimate for an arable soil. Estimates of soil biomass obtained by indirect methods are in agreement with the values given above. Bowen and Cawse (1964) measured organic carbon, released into soil solution following gamma radiation, which they believed arose from lysis of dead microbial cells. The values obtained corresponded to 340 and 1040 p.p.m. dry weight of microorganisms for two agricultural soils classified as light and heavy loams respectively. Jenkinson (1966) calculated values for the biomass in soils from the Broadbalk Continuous Wheat Experiment at Rothamsted based on the flush of CO_2 when the soils were treated with chloroform vapour. The values ranged from 330–620 p.p.m. carbon corresponding to 700–1300 p.p.m. dry weight of organisms. Taking an average value of 2% of dry weight for the phosphorus content of the soil microflora and fauna (Bowen 1966), the amount of phosphorus contained in the soil biomass, exclusive of plant material, could range from 5 p.p.m. phosphorus for an uncropped soil to >50 p.p.m. phosphorus for a fertile soil under legumes.

It seems reasonable to expect that desiccation will have a similar effect on vegetative cells of the microflora and soil animals to that observed for wheat roots, resulting in an increased water solubility of phosphorus contained within their biomass. Thus, in an arable or pasture soil which has been completely dried to a depth of 10 cm or more, there will be a pool of phosphorus in excess of 5 p.p.m. which can enter soil solution when the soil is re-wet. The actual amount of phosphorus in solution and its subsequent fate will be determined by plant density, the chemical and physical properties of the soil, and rainfall.

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VII. References

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