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PHOSPHORUS TRANSFORMATIONS DURING PLANT DECOMPOSITION

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The object of the following work was to follow the transformations undergone by plant phosphorus (a high proportion of which is inorganic) during decomposition in order to gain further insight into the conditions governing the nature and amount of the residual phosphate compounds reaching the soil. The problem is simplified by the fact that with most plant materials the initial supply of inorganic phosphorus is adequate for both the metabolic and synthetic processes of the microbial population throughout decomposition. Changes in microbial phosphorus during the development and decline of the population are therefore largely involved rather than mineralisation of plant organic compounds, which occurs at a slow rate. A difference may therefore be drawn between organic nitrogen and organic phosphorus with regard to decomposition and mineralisation. The amount of inorganic nitrogen in plant material is usually small so that mineralisation of organic nitrogen (which readily occurs with most plant materials) is essential for the decomposition process. On the other hand supplies of inorganic phosphorus are usually adequate for microbial needs during decomposition and they are not dependent on the mineralisation of organic phosphorus. The important factor in plant decomposition-phosphorus relationships is the extent to which the inorganic phosphorus initially present is converted to microbial organic phosphorus and how far this is recoverable (through dephosphorylating processes) as inorganic phosphorus on further decomposition or when decomposition is virtually complete.

The decomposition of various plant materials was followed in the macro-respirometer (Birch and Friend [4]) with reference

to changes in the various fractions detailed later. The results indicated that further experiments using glucose (instead of plant material) and various amounts of inorganic nitrogen and phosphorus salts should be carried out. The relevant part of this study is mentioned in the present paper.

EXPERIMENTAL

Materials

Finely ground (20 mesh) dry plant materials were selected which covered a range of phosphorus contents (Table 1).

TABLE 1

Samples used in the experiments

Material	Lab. No.	% N	A % P (total)	B % P (inorganic)	$\frac{B \times 100}{A}$	Group
Kikuyo grass . . .	33	2.92	0.228	0.196	86.0	1
Sorghum leaf . . .	756	3.07	0.348	0.273	78.0	Young
Rhodes grass. . . .	35	2.42	0.192	0.172	87.0	
Star grass.	2436	1.60	0.081	0.050	62.0	
,, ,, ...	2449	1.78	0.171	0.086	50.0	2
,, ,, ...	2457	1.40	0.181	0.085	47.0	Mature
Sorghum leaf . . .	745	3.84	0.532	0.323	61.0	

Methods

Four hundred milligrams of plant material were added to 50 g of fine (40–100 mesh) acid-washed, phosphorus-free sand in an 8-oz. wide-mouth sample bottle. Six millilitres of water were then added and the bottle was connected to the remainder of the respirometer unit and incubated at 25°C. A solution of sodium hydroxide was included in the bottle to trap evolved carbon dioxide. Twelve replicates were prepared in this way. After two days decomposition one of the bottles was withdrawn and the contents analysed. Other replicates were taken after 3, 4, 5, and 6 days decomposition and subsequently at longer intervals. An additional replicate was prepared for analysis immediately after adding the water (zero decomposition time).

On removal of the sample the amount of carbon mineralised was determined titrimetrically (Birch [3]). The sample was then divided into two. One half was placed immediately while moist in a vacuum desiccator containing chloroform, and left under reduced pressure in contact with the vapour for 24 hours. The other half was divided into four equal parts. One of these (A) was shaken with 25 ml of water in a wrist-action shaker for 1 hour and another (B) with 25 ml of *N* sulphuric acid. The third portion (C) was refluxed for 1 hour with 25 ml *N* sulphuric acid while the fourth portion

(D) was treated with 25 ml of *N* sulphuric acid and kept at 100°C in a sealed tube for 5 days.

After the foregoing extractions (A), (B), and (C) were filtered and an aliquot (2.5 or 5.0 ml depending on the plant material) added to 2 ml of Truog's [14] reagent diluted with about 30 ml of water (to avoid contact of the aliquot with strong acid with possible hydrolyses of any organic phosphorus extracted under (A) or (B)) in a 50-ml graduated flask. After making to the mark with water the phosphorus content was determined photometrically 14 minutes after the addition of 5 drops of 1% stannous chloride solution in 2 *N* hydrochloric acid. The aliquots from treatment (B) and (C) were neutralised with 1 : 1 ammonia solution, using a 0.5% aqueous solution of *p*-nitrophenol as indicator, before adding to Truog's reagent. With treatment (D) 3-ml aliquots were withdrawn after 5 days and phosphorus determined as for (B) and (C). The chloroform-treated samples were similarly divided into four parts and put through the same extraction procedures. The complete procedure described above was applied to some, but not all, of the samples.

Nature of the phosphorus fractions

(A) Water extracts inorganic phosphorus. At zero decomposition time it gives the amount of inorganic phosphorus in the plant material. The difference between this amount and that subsequently extracted after different periods of decomposition is equivalent to the organic phosphorus content of the micro-organisms (Caldwell and Hinshelwood [8]).

(B) Cold acid extracts inorganic phosphorus and, in addition, adsorbed inorganic phosphorus in the microbial cells. Caldwell [7] estimated that only about 10 per cent of bacterial phosphorus was present in inorganic form in the cells of *Bact. lactis aerogenes*. In the present studies the results obtained with cold-water and cold-acid extraction were often similar. Reference to the data for cold-acid extractions is therefore subsequently made only when there was a divergence in values between the two methods. This may be due to hydrolysis of glucose-1-phosphate which, according to Anderson [1] is hydrolysed by mild acid treatment.

(C) The amount of inorganic phosphorus obtained after refluxing 1 hour with boiling *N* sulphuric acid minus the amount of water-soluble inorganic phosphorus measures the amount of acid-labile phosphorus. The components of this fraction were not determined but according to Caldwell and Hinshelwood [8] they probably consist of ribose-3 phosphate, adenosine-3 phosphate and guanosine-3 phosphate. These are phosphorylated metabolic intermediates.

(D) Treatment of the samples for five days at 100°C with *N* sulphuric acid resulted in the almost complete hydrolysis of all the microbial organic phosphorus (including acid-labile) to inorganic phosphorus. The amount of acid-stable phosphorus may therefore be obtained by the difference between organic phosphorus and acid-labile phosphorus. Included in this group are phospholipoids and nucleic acids (Caldwell [7]).

Treatment of the samples with chloroform vapour prior to extraction measures changes in enzyme activity during decomposition since bacteria, which were found to be mainly operative throughout decomposition, are susceptible to this treatment. Enzymatic activity following death in this way is particularly marked during the early stages of decomposition, especially with the Group-1 samples, and brings about the hydrolysis of both acid-labile and acid-stable compounds to inorganic phosphorus. The chloroform experiments also appear to distinguish between enzyme systems responsible for the hydrolysis of both acid-labile and acid-stable organic phosphorus to inorganic phosphorus and for the conversion of acid-stable to acid-labile phosphorus. Further reference to this aspect of the work is made in Expt. 4.

Experiment 1. Changes in the amount of inorganic phosphorus during decomposition

Changes in water-soluble inorganic phosphorus for 7 samples are shown (smoothed out) in Fig. 1. Three patterns are evident

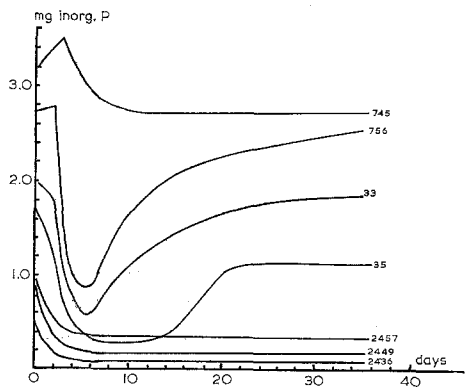

Fig. 1. Uptake and release of inorganic P during decomposition of various plant materials (1 g).

namely (1) almost complete recovery of the inorganic phosphorus initially utilised by the micro-organisms, (2) partial recovery, and (3) no recovery. These patterns were duplicated with glucose and inorganic salts (Expt. 5). The course of carbon mineralisation is shown in Fig. 2 where curve *A* is typical of plant materials

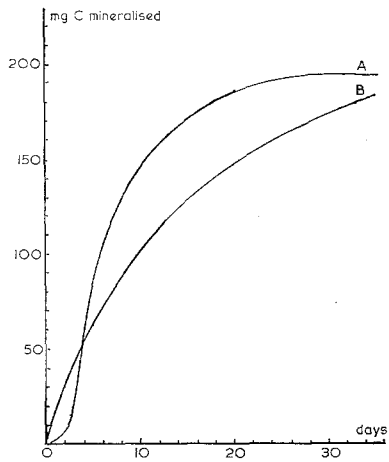

Fig. 2. Course of organic carbon mineralisation during decomposition of young (curve A) and mature (curve B) plant materials (1 g).

(Group 1, Table 1) giving a complete or partial recovery of inorganic phosphorus and curve *B* is typical of those giving no recovery (Group 2, Table 1). Group-1 samples show little decomposition during the first two days. Thereafter there is a marked increase in decomposition, the rate subsequently falling to a very low level. Group 2 exhibit a more uniform rate of decomposition from the start. The magnitudes of decomposition do not, in general, differ greatly but Group-1 samples generally decompose to a greater extent than the Group-2 samples over the 5 week period. It appears that the difference between decomposition curves *A* and *B* is due to differences in substrate availability rather than to microbiological differences since similar bacterial populations were involved in all instances throughout decomposition.

The loss of structural identity and build-up of ammonia during decomposition of the Group-1 samples (behaviour not shown by Group 2) indicates that Group 1 is concerned with young plant

material (supplying readily utilisable substrate) and Group 2 with mature material the components of which are more resistant to decomposition which is, therefore, more prolonged.

A feature of interest in Fig. 1 is the curve for Sample 745. In spite of the relatively high organic phosphorus content of this sample there is no evidence of mineralisation of plant organic phosphorus after 5 weeks and the form of the curve is not dissimilar to those for the remaining Group-2 samples of much lower phosphorus content. The inorganic phosphorus status during decomposition appears to be more closely related to substrate availability than the amount of organic phosphorus initially present in the plant.

Experiment 2. The effect of chloroform vapour on the amount of inorganic phosphorus extracted

Samples withdrawn at various stages of decomposition were halved. One half was extracted immediately with water and the other half after 24 hours exposure to chloroform vapour. In this way the amount of microbial

TABLE 2

Hydrolytic effect on microbial organic phosphorus after killing the cells with chloroform

Decomposition period (days)	Sample							
	33		756		49/57 †		2457	
	O.P.*	% Rec.**	O.P.	% Rec.	O.P.	% Rec.	O.P.	% Rec.
2	0.132	Nil			0.702	58	0.320	28
3	1.245	79	0.924	58	0.649	53	0.333	35
4	1.351	84	2.540	70	0.636	35	0.384	23
5	1.324	76	2.001	90	0.834	49	0.384	23
6	1.086	63	2.052	80	0.702	25		
7	1.324	48			0.768	34		
9			1.462	20	0.768	45	0.538	19
10			1.591	11	0.795	43		
12							0.474	8
16	0.556	Nil			0.689	6		
17			0.821	4			0.538	12
20	0.741	21	0.949	0			0.513	20
21					0.874	26		
25			0.949	0			0.589	17
26	0.586	18						
27					0.795	13		
31			1.155	0			0.437	6
37	0.238	Nil			0.662	0		

* O.P. = mg microbial P based on 1 g plant material taken initially.

** % Rec. = percentage of O.P. hydrolysed to water-sol. inorg. P after killing the cells.

† 49/57 = mixture of Samples 2449 (2 parts) and 2457 (1 part).

organic phosphorus enzymatically hydrolysed to inorganic phosphorus after killing the cells can be calculated by difference. Table 2 shows the results for two Group-1 samples and two Group-2 samples.

The data in Table 2 may be considered in conjunction with the effect of chloroform shown diagrammatically in Figs. 3, 4, and 5. During the early stages of decomposition a large viable population exhibiting high enzyme activity is operating. Death of the young population (through chloroform) causes reversible reactions with enzymatic dephosphorylation of organic to inorganic phosphorus. The sudden drop in enzyme activity shown by Samples 33 and 756 more or less coincides with the end of the active decomposition period. At this stage the cells are senile, the enzymes decay, and the chloroform effect is small. Continuity of enzyme activity depends on the production of new cells, and the less dramatic decline in the effect of chloroform on dephosphorylation with Samples 2457 and 49/57 indicates the continued presence of new cells because of the more prolonged availability of substrate obtaining with these samples.

It should be mentioned here that plant material moistened and immediately exposed to chloroform vapour always gives a slightly greater amount of water-soluble inorganic phosphorus than non-exposed samples. This may be due to plant enzyme activity and/or diffusion of inorganic phosphorus from the plant during the 24 hours exposure to chloroform. The magnitude of the chloroform effect for zero decomposition time has, however, been subtracted from the magnitude of the effect for subsequent decomposition periods in arriving at the data given in Table 2, which, therefore, refers entirely to the effect of chloroform on bacteria. Should the chloroform effect for zero decomposition time not be operative once decomposition starts the percentage recoveries in Table 2 would be greater throughout particularly with Samples 49/57 and 2457 where the correction is applied to smaller absolute values for the chloroform effect then with Samples 33 and 756, thereby leading to a more marked reduction in the precentage-recovery data.

The amount of inorganic phosphorus released following chloroform treatment may, on the basis of the foregoing experiment, find wider application as an index for the presence of viable bacterial cells.

Experiment 3. Effect of prolonged decomposition on the recovery of inorganic phosphorus

In this experiment 6 of the samples listed in Table 1 were incubated for 3 months, and the amounts of inorganic phosphorus determined by water extraction before and after decomposition (no intermediate determinations were made). The results are given in Table 3. The slight differences between the values in column 3 (Table 3) and column 5 (Table 1) are attributed to sampling errors and differences in vibration rates during extraction.

TABLE 3

Recoveries of inorganic P after 3 months decomposition. (On the basis of 1.0 g of plant material initially taken)

Sample No.	mg C mineralised	Inorganic P, mg	
		Before decomp.	After 3 mths. decomp.
756	294	2.754	2.448
35	292	1.648	0.761
33	238	1.929	1.697
745	222	3.372	3.582
2436	204	0.504	0.026
2457	205	1.028	0.437

There is no evidence that even on prolonged decomposition plant organic phosphorus is mineralised to give greater amounts of inorganic phosphorus than those initially present, though this must occur during the later slow process of humus decomposition.

Experiment 4. Interrelationships between the various phosphate fractions during decomposition

Changes in the amounts of the various fractions described at the start of the experimental section were followed with Sample 756 (Group 1) and 2457 (Group 2). Sample 33 (Group 1) was also studied with the exception of the 5-day hydrolytic period with N sulphuric acid at 100°C. The results obtained for this period with Sample 756 are, however, applicable in interpreting the results with Sample 33.

Sample 756. During the first 2 days there was a small increase in the amount of inorganic phosphorus which together with the main supply was subsequently utilised by the bacteria when decomposition started. Day 2 is therefore taken as the starting point in this instance. The amounts of acid-labile, acid-stable,

and chloroform-labile phosphorus were corrected throughout to allow for the amounts initially present in the undecomposed material.

The data are plotted in Fig. 3 to show the amount of bacterial organic phosphorus (Graph 1) present during the various stages of decomposition (assuming 1 g of sample initially taken). The amount of acid-labile phosphorus is shown by Graph 2. The difference between Graphs 1 and 2 represents acid-stable phosphorus. The amount of phosphorus hydrolysed after 5 days with *N* sulphuric acid at 100°C corresponded almost exactly, throughout decomposition, with the bacterial organic-phosphorus content. Since it is therefore, practically synonymous with Graph 1 it is not shown in the figure. The broken lines (Graphs 4 and 5) show the amount of inorganic phosphorus obtained when the samples were exposed to chloroform vapour prior to extraction with water and hot *N* sulphuric acid (1 hour) respectively.

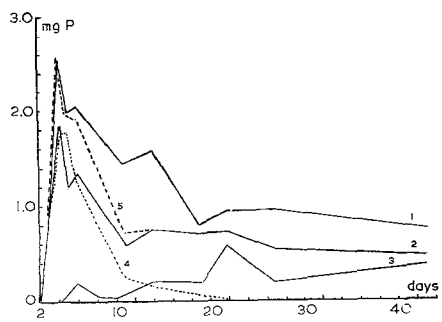

Fig. 3. Sample 756. P-transformations during decomposition. Graph 1, microbial organic P. This is made up of acid-labile P (Graph 2) and acid-stable P (Graph 1–Graph 2). Cold-acid-extractable P (Graph 3) is part of the acid-labile fraction. Graphs 4 and 5 show the amounts of inorganic P recovered from the organic P when the samples are exposed to chloroform vapour prior to extraction with water (Graph 4) and hot acid (Graph 5).

Fig. 3 shows that an initial rapid build-up of organic phosphorus is followed by a decline as dephosphorylation to inorganic phosphorus takes place. The close parallelism between Graphs 1 and 2 during the first 12 days indicates that dephosphorylation largely involves hydrolysis of the acid-labile organic phosphorus compounds. On the twelfth day a change in the dephosphorylation process

takes place and inorganic phosphorus starts to be formed at the expense of the acid-stable organic phosphorus, while the amount of acid-labile phosphorus declines slowly. Hydrolysis of the acid-stable forms may be due to lysis of the cells formed earlier as a result of which contact is established between dephosphorylating enzymes and acid stable compounds formerly separated from these. An alternative possibility is mentioned in the discussion. By the 17th day practically all the organic phosphorus is in the acid-labile form. Thereafter there is apparently some conversion of acid-labile to acid-stable phosphorus after which the latter slowly mineralises. By the 41st day the residual organic phosphorus contained approximately 65 per cent acid-labile phosphorus and 35 per cent acid-stable phosphorus. The amount of cold-acid-extractable phosphorus is negligible during the early stages of decomposition but shows a tendency to increase during the later stages.

Treatment of the sample with chloroform vapour prior to extraction with water resulted during days 2 to 6 in the recovery of inorganic phosphorus in amounts almost equal to the acid-labile values (compare Graphs 2 and 4) after which the effect of chloroform declines noticeably. When the chloroform-treated samples were hydrolysed for 1 hour with boiling *N* sulphuric acid the amount of inorganic phosphorus recovered was greater than that from the non-chloroform treated samples (*cf* Graphs 5 and 2) and between the 2nd and 6th day accounts for practically all the bacterial organic phosphorus. The effect was negligible by the ninth day. Enzyme activity following chloroform treatment during the early stages converted acid-stable to acid-labile phosphorus. There is therefore a period during the early stages of decomposition when enzyme activity following chloroform treatment can convert acid-labile to inorganic phosphorus, and acid-stable to acid-labile phosphorus. Conversion of acid-stable to inorganic phosphorus can also occur as shown with sample 33.

Sample 33. This sample, after decomposition, gave an almost complete recovery as inorganic phosphorus of the bacterial organic phosphorus initially formed. The amounts of the various fractions present at progressive stages of decomposition are shown in Fig. 4.

The overall changes during the early stages of decomposition are similar to those for Sample 756. An initial rapid build-up of

organic phosphorus (Graph 1) is followed by its dephosphorylation involving first the conversion of acid-labile forms (Graph 2) and then of acid-stable forms, (Graph 1 minus Graph 2) to inorganic phosphorus. A marked decline in acid-stable phosphorus starts only on the 20th day. From the 16th to the 26th day there is an increase in acid-labile phosphorus when secondary decomposition may occur resulting in renewed bacterial synthetic processes. Thereafter this again declines giving rise to inorganic phosphorus the amount of which is further enhanced by steady dephosphorylation of acid-stable phosphorus (Graph 1 minus Graph 2). The final composition of the residual organic phosphorus, namely about 75 per cent acid-labile and 25 per cent acid-stable is similar to that for sample 756.

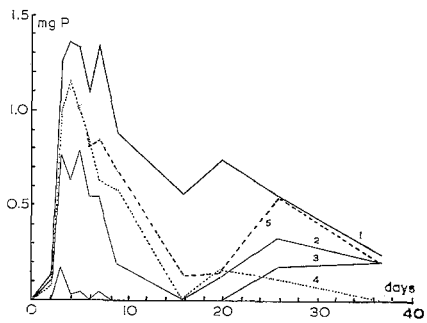

Fig. 4. Sample 33. Gramphs 1 to 5 as under Fig. 3. Graphs 4 and 5 are synonymous up to day 6.

The amount of cold-acid extractable phosphorus (Graph 3) is negligible until the 20th day after which, as with Sample 756 there is an increase until, at the end of decomposition, the amount is equal to acid-labile phosphorus. This reflects the presence of easily hydrolysable organic phosphorus compounds (possibly glucose-1-phosphate) rather than microbially adsorbed inorganic phosphorus (see under Experimental) since it is unlikely that about 75 per cent of bacterial organic phosphorus would be inorganic. The whole of the acid-labile phosphorus with sample 33, and part of it with Sample 756, is therefore in a readily hydrolysable form when decomposition is virtually complete.

Graphs 4 and 5 refer to samples exposed to chloroform vapour prior to extraction. Graph 4 shows the amount of inorganic phos-

phorus extracted with water and Graph 5 the amount produced after hydrolysis with *N* sulphuric acid at 100°C for 1 hour. Graphs 4 and 5 for the first 6 days are almost identical and show that chloroform treatment has effected the enzymatic hydrolysis of both acid-stable and acid-labile phosphorus to inorganic phosphorus, and that a considerable proportion of the organic phosphorus is susceptible to this treatment.

After the 6th day enzymatic activity following chloroform treatment becomes increasingly ineffective and is negligible by the 16th day. After the 20th day there is a recovery in enzyme activity involving the conversion of acid-stable to acid-labile forms. This may reflect secondary decomposition (consequent upon lysis of bacterial cells produced earlier) which would give rise to a newly developing population susceptible to exposure to chloroform and containing active enzyme systems.

Sample 2457. This sample gave little recovery of inorganic phosphorus even after three months decomposition (see Table 3). The distribution of the various fractions during decomposition is shown in Fig. 5.

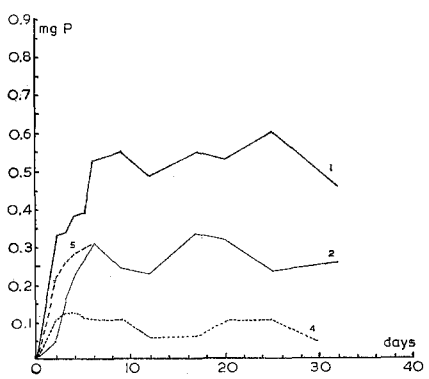

Fig. 5. Sample 2457. Graphs 1 to 5 as under Fig. 3. Zero values for Graph 4.

There was a marked build up of microbial organic phosphorus during the first six days after which approximately equal amounts of acid-labile phosphorus (Graph 2) and acid-stable phosphorus (Graph-1 values minus Graph-2 values) were present. All the organic phosphorus present at the various stages of decomposition was hydrolysed during 5 days with hot *N* sulphuric acid. No cold-

acid-extractable phosphorus in excess of water-soluble inorganic phosphorus was obtained throughout the decomposition period.

The absence of any marked fluctuations in the various fractions, such as was found with samples 756 and 33, after the sixth day, indicates a steady state in the microbial population. It appears that after the initial build-up of bacterial organic phosphorus any inorganic phosphorus subsequently produced through dephosphorylation is utilised by newly developing bacteria, this process continuing as long as there is available substrate to the decomposed. The persistance of slowly decomposable substrate with mature plant material was mentioned earlier.

When the samples were treated with chloroform prior to extraction with water a small proportion of the bacterial organic phosphorus was dephosphorylated to inorganic phosphorus (Graph 3), which remained fairly constant throughout the decomposition process. This indicates the presence of viable cells at a relatively low and constant level and supports the hypothesis outlined in the previous paragraph namely, that as old cells die off, the inorganic phosphorus released is utilised by newly developing bacteria which in turn die off and release inorganic phosphorus, this process continuing as long as substrate is available for decomposition. It is probable that when the substrate is eventually exhausted dephosphorylation of bacterial organic phosphorus according to the processes found for Samples 33 and 756 will occur.

When the samples were exposed to chloroform vapour and then extracted with hot acid the amount of acid-labile phosphorus obtained (Graph 4) during the first four days was greater than from the non-chloroform-treated samples after which there was no difference between the two treatments. If the effect of chloroform was to bring about enzymatic hydrolysis of acid-stable phosphorus Graph 4 would be above Graph 2 after the fourth day. Hence it appears that only prior to the fourth day are acid-stable forms enzymatically hydrolysed on death of the cells to acid-labile forms.

Experiment 5. Inorganic phosphorus fluctuations during glucose decomposition

Solutions were prepared such that 6.0 ml contained the amounts of carbon (as glucose), nitrogen (as ammonium sulphate), and phosphorus (as potassium dihydrogen phosphate) shown in Table 4. Each solution contained, in addition, 2.4 mg of magnesium sulphate.

TABLE 4

Solutions used for glucose decomposition experiments							
Treatment No.	mg C	mg N	mg P	C : N	C : P	N : P	glucose decomposition (32 days), %
1	96	1.2	1.080	80	89	1.1	97.0
2	96	9.6	0.216	10	444	44.5	81.6
3	96	9.6	0.864	10	111	11.0	98.8

Each of the solutions was added to 50 g sand plus 0.1 g soil (as inoculum) and incubated at 25°C in the respirometer. Two-gram samples of each treatment were subsequently withdrawn at intervals for the determination of water-soluble inorganic phosphorus. The effect of exposure to chloroform vapour (prior to extraction) on the amount of inorganic phosphorus extracted and the amount of glucose decomposed at various stages, were also followed.

Fungi were found to be largely involved in the decomposition processes and, in contrast to bacteria, gave little extra inorganic phosphorus after chloroform treatment. Work here indicates that fungi are not susceptible to this treatment.

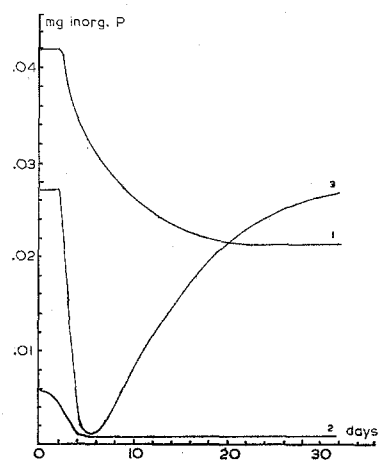

Fig. 6. Inorganic-P fluctuations during glucose decomposition. P as mg P per 2-g sample removed at intervals. Curves 1 to 3 refer to treatments in Table 4.

Fig. 6 shows no recovery of inorganic phosphorus with Treatments 1 and 2 where, because of low amounts of N and P respectively early decomposition is slower than with Treatment 3 and more

prolonged. This result is parallelled by Group-2 plant samples where decomposition is also prolonged, but in this instance because of the poor availability of the substrate.

Treatment 3, in which initial decomposition was rapid resulted in an almost complete recovery of inorganic phosphorus. Here it appears, as with the Group-1 plant samples, that a large microbial population is initially built up and, having utilised most of the substrate, dies off with release of inorganic phosphorus as lysis occurs. The high proportion of glucose decomposed even under low inorganic-phosphorus conditions is also noteworthy (Table 4).

DISCUSSION

The foregoing work demonstrates that recovery of microbial organic phosphorus, synthesised from plant inorganic phosphorus during the early stages of decomposition, depends largely on rapid decomposition and exhaustion of substrate leaving a large population to undergo autolysis with the release of inorganic phosphorus through enzymatic dephosphorylation processes. Substrate causing such behaviour is afforded by young plant material. With mature material the microbial population built up initially is smaller and the more prolonged supply of less readily available substrate maintains a fairly uniform cycle of microbial growth and decay in which any microbial organic phosphorus mineralised is used again. With both young and mature material however no pronounced mineralisation of plant organic phosphorus appears to take place during decomposition periods up to three months.

Chang [9] states that the formation and mineralisation of organic phosphorus complexes seem to depend on the amount of available energy-supplying materials, which are themselves influenced more by the age than the nature of the plant. He also remarks that when the materials which supply available energy become exhausted the micro-organisms attack complex organic phosphorus compounds including those originally present in the plant. There is however no evidence with the above samples that this stage is reached even after three months, when decomposition is virtually complete. It is possible that mineralisation of plant organic phosphorus is masked by microbial uptake of the inorganic phosphorus produced. Maximum mineralisation of plant organic phosphorus would,

however, be expected to occur during the early stages of decomposition when microbial activity is high, yet when the bacterial cells were killed during this period no more inorganic phosphorus was recovered than was present before decomposition, indicating that little or no mineralisation of organic phosphorus had occurred. From the plant decomposition studies and Experiment 5 (which shows similar patterns of behaviour although no organic phosphorus was originally present) it appears that phosphorus transformations involving interactions between bacteria and the inorganic phosphorus initially present are of greater significance than the mineralisation of plant organic phosphorus which appears to occur only slowly.

Kaila [10] using natural organic materials found an increase in organic phosphorus over a three-month decomposition period with rye straw and wheat roots, and little change with horse dung. Mineralisation of organic phosphorus initially present in the material occurred however with wheat bran, fungal material, cattle manure and green fodder, which contained 0.78, 0.27, 0.22, and 0.15 per cent organic phosphorus respectively. The only plant material containing a comparable amount of organic phosphorus in the present work was Sample 745 (0.21 per cent) which, from Table 3, may have undergone slight mineralisation after three months decomposition. In general with plant material we are dealing with organic-phosphorus contents lower than this, but decomposition is not hindered since the supply of inorganic phosphorus is sufficient and can, if necessary, be re-utilised during decomposition as it becomes available from dead micro-organisms. In this connection it may be noted that Caldwell and Hinshelwood [8] found that the inorganic phosphate in the medium had to become almost completely exhausted before any serious effect on the growth rate of *Bact. lactis aerogenes* could be detected. (*cf* Col. 8, Table 4).

Changes in the various phosphate fractions constituting the bacterial organic phosphorus are described in Experiment 4, in which two stages are apparent in the dephosphorylation of microbial organic phosphorus namely (a) hydrolysis of acid-labile phosphorus followed by (b) that of acid-stable phosphorus. According to Bartholomew and Goring [2] phosphorus release and cell decomposition (of the cell suspension used in their experiments) was in

part the result of autolysis and in part the result of decomposition by other micro-organisms. Possibly stage (a) above is related to autolysis and stage (b) to decomposition by a freshly developing population using the lysed products. This is to some extent supported, with Sample 33, by the reappearance of acid-labile and acid-stable forms of phosphorus, as well as by a response to chloroform vapour, such as would be expected with a newly developing population. Alternatively stage (a) may be due to internal changes in ageing cells and stage (b) to autolysis itself.

Bartholomew and Goring [2] found that decomposition of the cells resulted in some breakdown of all the organic phosphorus complexes. A similar result was found here and in one instance (Expt. 4, Sample 33) almost complete dephosphorylation of both acid-stable and acid-labile compounds occurred. After about five weeks decomposition with sample 33 and 756 only 21 and 31 per cent respectively of the maximum amount of microbial organic phosphorus synthesised earlier remained, consisting of approximately 70 per cent acid-labile phosphorus and 30 per cent acid-stable phosphorus. The declining portions of Graph 1 (Figs. 3 and 4) are of an asymptotic nature and it appears that with time and under uniform moist conditions both these forms would eventually be mineralised. With Sample 2457 after 5 weeks decomposition about 81 per cent of the maximum amount of microbial organic phosphorus formed was still present, made up of approximately equal amounts of acid-labile and acid-stable phosphorus. These conditions are likely to persist as long as substrate is available but eventually transformations of the kind found in the decomposition of Samples 33 and 756 are likely to occur. Hence the plant inorganic phosphorus tied up as microbial organic phosphorus during decomposition may all be recovered as inorganic phosphorus (provided that death and lysis of the micro-organisms follows an uninterrupted and normal course) however difficulty hydrolysable by chemical methods these compounds may be. These experiments were however carried out in a sand medium whereas in soil protection from decomposition through adsorption of the organic phosphorus compounds or of the enzymes may afford some protection of these potentially mineralisable compounds.

The high proportion of phytin found by Bower [5] in the organic-phosphorus fraction of soils is of plant origin (since it is not a

constituent of micro-organisms) and further supports the observations made above that plant organic phosphorus compounds are little mineralised during plant decomposition presumably because of their resistance to decomposition. The overall result of plant decomposition appears to be, from the phosphorus aspect, the formation of residual compounds consisting of largely unchanged plant organic phosphorus together with acid-stable and acid-labile microbial organic phosphorus in amounts and proportions depending on the material decomposed (young or mature), the stage at which decomposition stopped and, under natural conditions, the influence of the soil.

There is little in the literature on the chloroform effect described above, but Stickland [13] found that the rate of liberation of inorganic phosphorus after poisoning of the esterifying systems of yeast with urethane was of a similar order of magnitude to the rates of esterification. A similar result was obtained here during the early stages of decomposition when exposure of the cells to chloroform vapour for 24 hours was sufficient to convert to inorganic phosphorus most of the organic phosphorus formed during the previous day (Figs. 3 and 4).

The conversion of inorganic to organic phosphorus during substrate utilisation, followed by dephosphorylation, is of wide occurrence. According to Brodie and Gray [6] the coupled oxidative phosphorylation observed with the bacterial system is similar to that in mammalian tissue, and utilisation of inorganic phosphorus probably involves the same type of mechanism. Macfarlane [11] found with yeast that when the free sugar had disappeared there was a reappearance of inorganic phosphorus in the cell. Mann [12], dealing with the phosphorus metabolism of moulds, found that mould fungi adsorb ortho phosphate aerobically and convert it to a number of organic compounds a large proportion of which is converted to acid-hydrolysable compounds. During autolysis of mycelium organic phosphorus compounds were decomposed and excreted into the medium as ortho phosphate. A similar result was obtained in Expt. 5, with Mixture 3, in the decomposition of which fungi were largely operative.

SUMMARY

During plant decomposition microbial organic phosphorus is rapidly formed from plant inorganic phosphorus. Subsequently with young plant material much of the microbial organic phosphorus is dephosphorylated and recovered as inorganic phosphorus. With mature material there was little recovery even after three months decomposition. Recovery depends on rapid utilisation and exhaustion of easily decomposable substrate leaving a relatively large microbial population to die and undergo lysis. Poor recovery is associated with less-decomposable substrate and the maintenance of a viable population.

In no instance was more inorganic phosphorus recovered after up to three months decomposition than before. Plants generally contain sufficient inorganic phosphorus for bacterial requirements during decomposition so that supplementary supplies obtained by mineralisation of plant organic phosphorus are unnecessary.

During dephosphorylation of microbial organic phosphorus acid-labile forms are first involved and then the acid-stable forms. Secondary decomposition possibly involving dead micro-organisms results in the resynthesis of both forms (though to a much smaller extent than during the early stages of decomposition). These however suffer further dephosphorylation and the residual products consist roughly of 70 per cent acid-labile and 30 per cent acid-stable phosphorus. With mature material the amount of organic phosphorus remains fairly constant during five weeks decomposition and consists of approximately equal parts of both forms.

Exposure of the samples at progressive stages of decomposition to chloroform vapour resulted in rapid dephosphorylation of the microbial organic phosphorus during the early stages of decomposition when microbial enzyme activity is high. Enzyme systems converting acidstable and acid-labile forms to inorganic phosphorus, and acidstable to acid-labile forms could be distinguished.

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