EFFECT OF CARBON ADDITIONS ON SOIL LABILE INORGANIC, ORGANIC AND MICROBIALLY HELD PHOSPHATE

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Investigations of the rate of P movement between soil inorganic, organic and biomass P compartments were carried out to clarify aspects of P cycling in soil systems. Organic carbon, as dried grass (33% C, 0.11% P) and cellulose (43% C), was added at a rate equivalent to 4000 kg organic material (OM) · ha^{-1} every 30 days for 9 mo to the Ap horizon of a Chernozemic Black soil kept at field capacity moisture content and 24±2°C. In a third treatment, cellulose was added at the same rate with P (20 kg · ha^{-1}) at KH_2PO_4. Approximately 39% and 22% of the P added in grass and with cellulose, respectively, was found in organic P forms after 9 mo incubation. The remainder was found in NH_4Cl-, NH_4F- and NaOH-NaCl-extractable P forms which constituted part of the labile inorganic P pool and could be extracted by an anion exchange resin. Increases of biomass P during the first 4 or 5 days of each incubation period after residue addition were found to average 12 μg P · g^{-1} in the first 3 mo incubation period. After this period, there was a smaller response in microbial P attributable to additions of grass or cellulose.

Des recherches ont été réalisées sur les échanges de P entre les éléments inorganiques, organiques et biotiques du sol, dans le but d'éclaircir certains aspects du cycle de P dans le sol. Du carbone organique, sous forme de foin séché (33% C, 0.1 % P) et de cellulose (43% C) ont été apportés, à un taux correspondant à 4000 kg de matière organique (m.o.) par dixième d'hectare tous les 30 jours pendant 9 mois, à l'horizon Ap d'un sol noir chernozémique maintenu à la capacité au champ et à une température de 24°C plus ou moins 2. Dans une troisième intervention, on a apporté la même quantité de cellulose avec 20 kg P par dixième d'hectare sous forme de KH_2PO_4. Environ 39 et 22%, respectivement, du P apporté par l'herbe et par la cellulose ont été recouvrés dans les composés organiques au bout de 9 mois d'incubation. Le reste se répartissait entre les formes de P extractible dans NH_4Cl, NH_4F et NaOH-NaCl qui constituent une partie du pool de P inorganique labile et qui pouvaient être extraites sur résine échangeuse d'anions. L'accroissement du P biotique dans les 4 ou 5 premiers jours de chaque période d'incubation après l'apport des résidus cellulosiques était en moyenne de 12 μg P par dixième de gramme, par dixième de mois dans les 3 premiers mois. Passé ce terme, on a constaté une réaction moins forte du P microbien imputable à l'incorporation de cellulose ou d'herbe.

Hannapel et al. (1964) showed that a significant proportion of the phosphorus (P) redistributed during incubation of a calcareous soil was closely associated with the cycling of bacteria and cell debris. However, most workers have attached more importance to the role of microorganisms in enzyme production than to their role as live phosphate "sinks." Halm et al. (1972) in a study of a cool season native grassland predicted from microbial biomass measure-

ment on the same site (Babiuk and Paul 1970) and from literature values of microbial P content that at any sampling date over the growth season more P ($g \cdot m^{-2}$) was contained in the soil microbial biomass than in the native vegetation. They recognized the importance of organic P flows in the supply and cycling of plant P but were unable to quantify the flows. A simulation model in which the major controls were the P solubility (recalculated daily from relationships to the labile inorganic pools), soil-water content, and rate of diffusion of P through soil (Stewart et al. 1973; Cole et al. 1977) predicted plant and decomposer uptake and turnover rates of the principal P compartments (Fig. 1) in the same grassland site.

Comparison of predicted P flows with actual field measurements pinpointed serious gaps in information on processes such as the rate of mineralization of organic P and the flows in and out of microbial forms. To fill these gaps, an investigation was undertaken to study the dynamics of P within the soil system. This investigation had the objectives of measuring: (a) the change in P forms (labile, inorganic and organic P fractions) with time in soils to which carbon sources were added every 30 days, and (b) to relate these transfers to microbial activity.

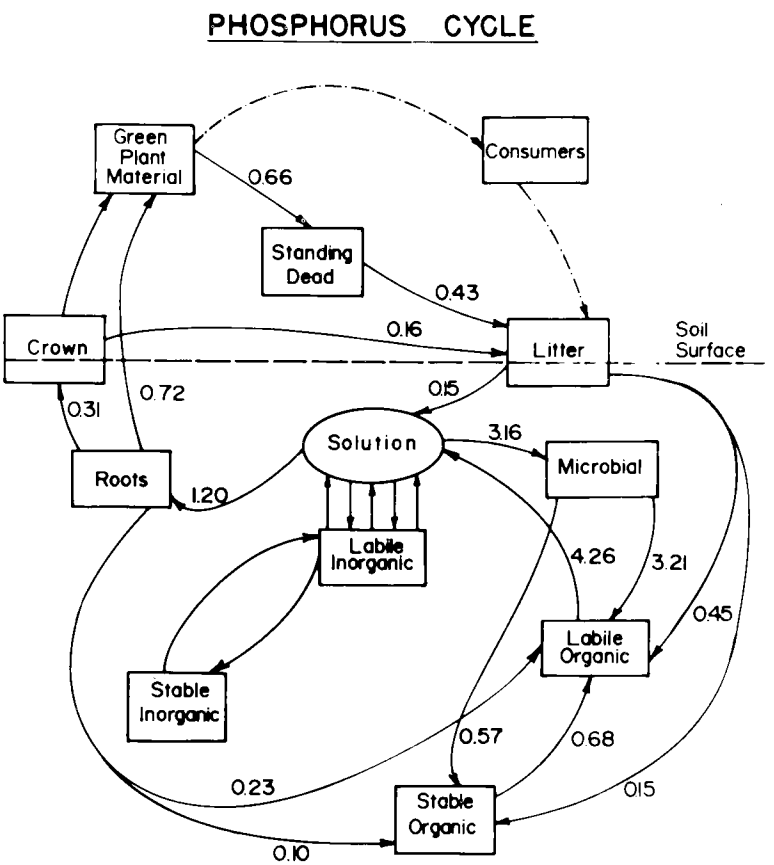

Fig. 1. Predicted flow of P ($g \cdot m^{-2}$) between components of a native grassland ecosystem (Cole et al. 1977).

MATERIALS AND METHODS

The Ap horizons of a Chernozemic Black soil (24% clay, 6.5% OM and *p*H 6.4) of the Oxbow Association (Ellis et al. 1965) were used in this experiment. The soil was air-dried at room temperature, ground to pass a 2-mm sieve and mixed very thoroughly prior to use. Carbon at a rate equivalent to 4000 kg OM · ha^{-} was added as ^{14}C labelled grass (32.8% C, 13.23 μCi · g^{-1}C, and 0.11% P) and as cellulose (43% C). In a third treatment, cellulose was added at the same rate with P as KH_2PO_4 at a rate equivalent to 20 kg P · ha^{-1}. In other treatments P was added without the addition of C and the original soil was incubated without additions of either C or P. Ammonium nitrate was used to adjust the C:N ratio of the cellulose treatment to 25 to 1. The uniformly ^{14}C-labelled, dried grass (*Bouteloua gracilis*, blue grama) (supplied by Dr. C. V. Cole, USDA-ARS Phosphate Laboratory, Fort Collins, Colo.) was finely ground in a Wiley mill and mixed prior to subsampling required amounts to add to soil. Required amounts of grass, cellulose, N and P were added and thoroughly mixed into soil every 30 days, then placed in pots and incubated at field capacity at 24±2°C. Algal growth was prevented by covering the surface of the pots with styrofoam beads. Moisture adjustments were made every second day.

Triplicate 1-kg soil samples were used. At the end of each incubation period, 50-g subsamples were removed from each larger sample prior to the further C or P additions. These subsamples were similarly treated with proportional additions of C and P, incubated in the same environmental conditions, but were sampled at different times relating to different decomposition stages in the 30-day incubation period. An extra series o f 50-g soil samples receiving the same treatments was incubated separately in air-tight desiccators containing known volumes of standardized NaOH solution. This enabled daily measurements of CO_2 evolution to be taken. Also as the latter series of incubation were started 5 days in advance of the main experiment, the rates of CO_2 production could be used to determine the times of sampling in the larger experiment. During every 30-day period, measurements of microbial biomass, CO_2 evolution, and P fractions, $NaHCO_3$ inorganic and organic extractable P, microbial P, solution P, resin-extractable P, and inorganic P fractions were taken at the time of the maximum decomposition rate of the added organic matter, as determined by CO_2 production, and at a later stage when the CO_2 production had levelled off and reached steady state conditions (Fig. 3). At the end of the 9-mo incubation period the organic P was fractionated as shown in Fig. 2.

The methods of analysis used in this study are presented in Table 1. Further details are given below for some of the more recently developed techniques.

Microbial P

Paired soil samples (air-dried <2 mm), one chloroform-treated and one untreated, were extracted at 1:20 soil solution ratio with 0.5 M

Table 1. Analytical methods used in this study

Methods	Reference
$NaHCO_3$-extractable P	
(i) Inorganic (P_i)	Olsen et al. (1954)
(ii) Organic (P_o)	Halm et al. (1972; Bowman and Cole (1978a)
Microbial P ($CHCl_3$ treatment + $NaHCO_3$ extraction after 6 days)	Bowman and Cole (1979)
Total, mineral and organic P	Saunders and Williams (method modified to use 2 N H_2SO_4; Anderson 1960)
Inorganic P fractions	Peterson and Corey (1966) modified for calcareous soils by Sadler and Stewart (1975)
Resin-extractable P	Amer et al. (1955)
Organic P fractions	Bowman and Cole (1978b)
Microbial biomass — bacteria	Babiuk and Paul (1970)
— fungi	Paul and Johnson (1977)
CO_2 evolution — NaOH absorbent in closed container	

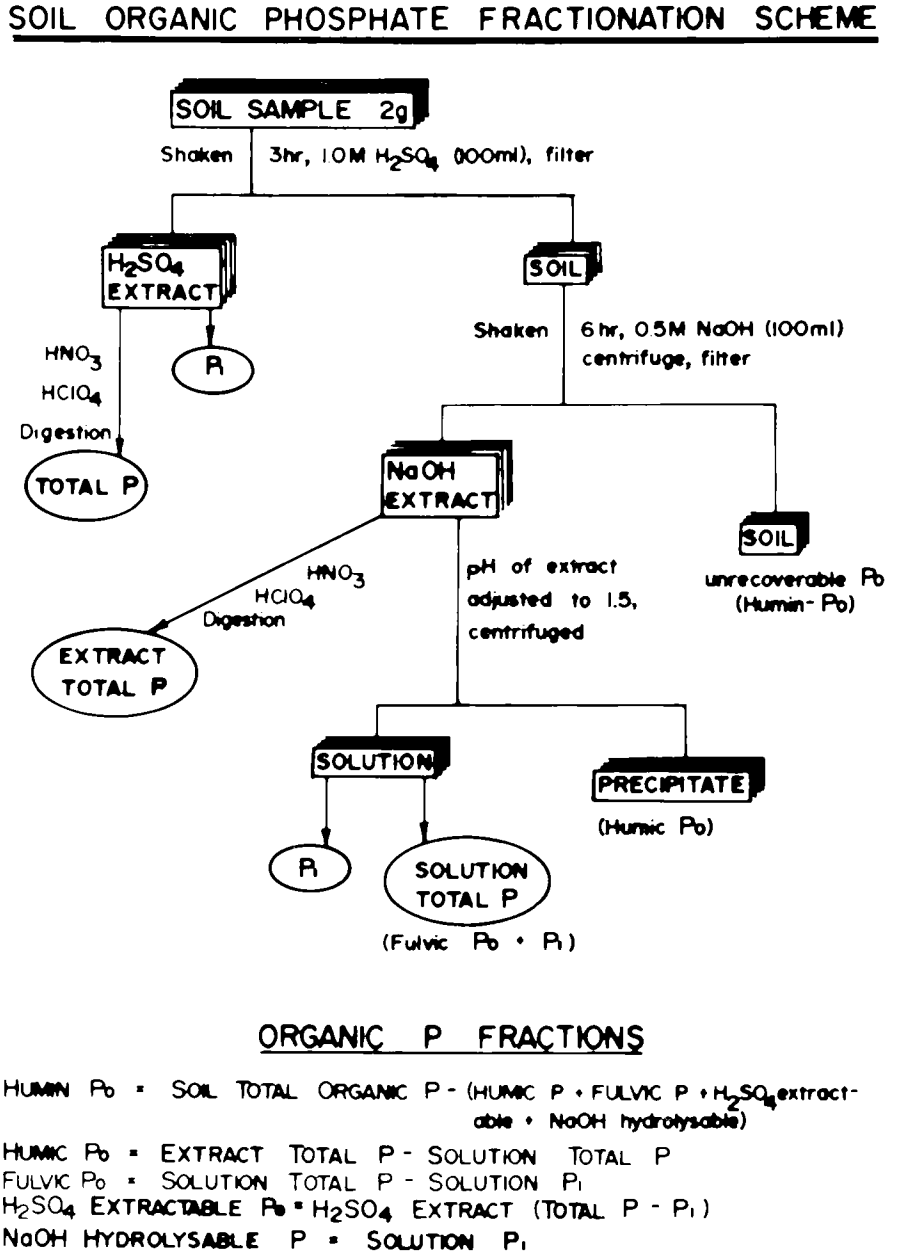

Fig. 2. Fractionation scheme used for soil organic P (Bowman and Cole 1978b).

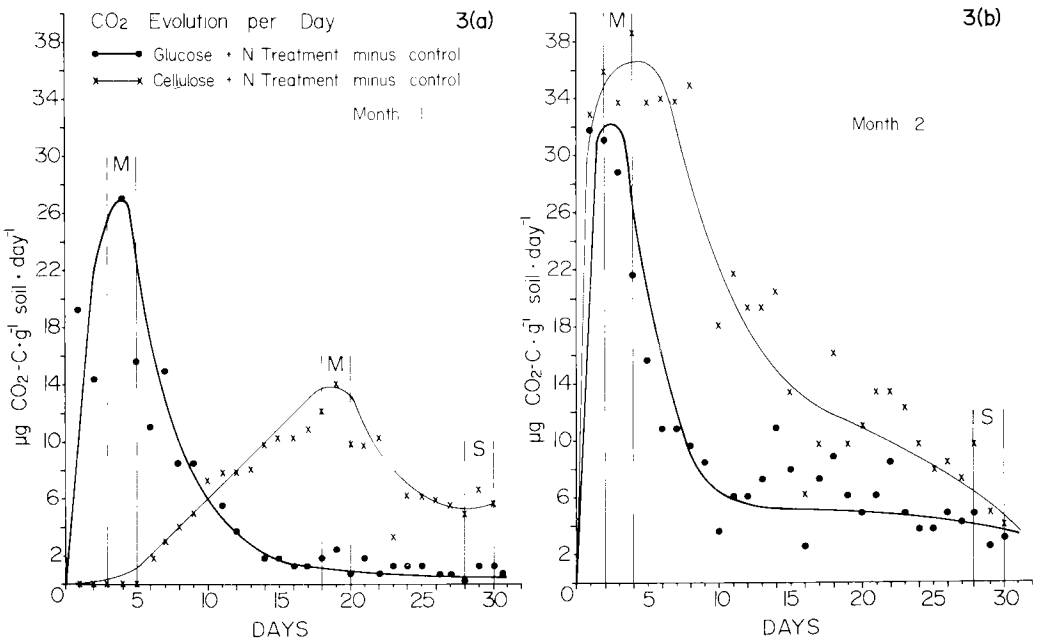

Fig. 3. A schematic representation of sampling periods for the first (a) and subsequent incubation periods (b). Daily measurements of the CO_2-C produced per gram soil per day gave the times of maximum decomposition (M) and steady state decomposition (S).

(*p*H 8.5) $NaHCO_3$ (Olsen et al. 1954). The difference in total P in the two extracts gave an estimate of microbial P. Liquid chloroform was applied directly to soils (1:1 wt/vol) for 30 min and then removed by aeration with a fan in a fumehood at 45°C. Chloroform-free dried samples were stored at room temperature for 6 days prior to extraction with $NaHCO_3$.

Microscopic biovolume measurements were converted to biomass using the moisture content and specific gravity of soil isolates. In this experiment two different values were used. One set of calculations utilized the common literature values of 80% H_2O and 1.1 specific gravity as used by Babiuk and Paul (1970). The other calculation used conversion values obtained by van Veen and Paul (1979) for microorganisms grown under moisture stresses commonly found in soil. These resulted in a multiplication of the bacterial biomass as calculated from literature values by 3.63. The fungal biomass was increased by a factor of 1.44. In the case of the fungi, the dry weight-specific gravity value of 1.44 could represent an organism with a specific gravity of 1.3 and an assumed moisture content of 76%.

RESULTS

Net changes in the inorganic and organic P fractions found in the soil following 9 mo incubation with and without grass, cellulose and P can be determined from the data presented in Table 2. The differences between the original P and the P in each of the fractions at the end of the 9-mo period showed that the total amount of P which was added in grass (18 μg P · g^{-1} soil) or added as fertilizer P (81 μg P · g^{-1} soil) over the 9 mo could be accounted for in all treatments except the cellulose + N + P treatment in which the recovery was 89%. The added P was present in both the inorganic and organic forms. Total inorganic P decreased 11% with the addition of cellulose plus N alone, did not change in the grass treatment but increased in all other treatments. Similarly, resin-extractable P and sodium bicarbonate-extractable inorganic P decreased in comparison to the control soil in the cellulose + N treatment, but both indexes of labile P were increased substan-

Table 2. Organic and inorganic fractionation data in the original soil and after treatment and 9 mo incubation. All results are expressed as $\mu g\ P \cdot g^{-1}$ oven-dry soil and are the average of three determinations of each of three replicates (± standard deviation)

	Original soil	Control soil	Grass-treated soil	(Cellulose + N)-treated soil	(Cellulose + N + P) treated soil	P-treated soil
			$\mu g \cdot g^{-1}$ soil			
			Total P			
Soil P	755±2	755±2	773±2	755±2	827±3	836±3
Inorganic P	276±1	281±2	284±2	255±0	324±1	353±4
Organic P‡	479	474	489	500	503	483
			Labile-P indexes			
Resin-extractable P	34.4±3.1	45.1±3.4	42.0±2.5	28.0±2.8	80.0±0.2	96.5±3.9
Solution P	0.6±0.1	0.7±0.1	1.3±0.2	0.3±0.0	3.2±0.0	5.5±0.1
$NaHCO_3$-extractable P	15.0±1.3	15.0±1.3	17.4±0.3	8.5±0.1	36.5±1.1	57.1±0.3
			Inorganic-P fractions†			
NH_4Cl-extractable P	3.8±0.0	6.1±0.9	4.0±0.2	2.3±0.9	13.7±0.5	20.5±0.3
NH_4F-extractable P	37.7±0.6	45.8±1.6	41.1±0.0	26.7±1.2	64.8±2.6	80.5±1.2
NaOH-NaCl-extractable P	32.6±0.4	33.3±0.2	33.8±1.6	29.1±0.7	41.9±0.2	46.8±0.2
			Organic-P fractions			
H_2SO_4-extractable P	100±3	103±3	112±3	108±3	114±4	99±9
Alkali-hydrolyzable P	53±3	36±3	39±2	38±0	37±1	40±1
Fulvic P	125±9	136±4	147±0	160±2	159±4	141±5
Humic P	115±6	119±6	105±2	106±8	102±7	115±2
Humin P‡	86	80	87	89	92	88

†Other inorganic P fractions RSP, NaOH-P and H_2SO_4-P not shown as relatively constant.
‡By difference.

tially through the addition of P. Examination of the data from inorganic P fractionation showed that most of the P immobilized by the addition of cellulose + N in the absence of added P came from the NH_4F-extractable P fraction and to a lesser extent from the NaOH-NaCl-extractable P. Most of the increases in inorganic P fractions were associated with the NH_4Cl-P, NH_4F-P and NaOH-NaCl-P extractable forms. These constituted part of the labile inorganic P pool that could be extracted with an anion exchange resin.

Total organic P increased with the addition of grass and cellulose. The changes accounted for 39% and 22% of the added P, respectively. Reductant soluble P (RSP), NaOH- and H_2SO_4-extractable P levels did not alter and therefore the data are not shown. The major changes with treatment were internal transfers in the amount of P found in H_2SO_4-extractable and NaOH-hydrolyzable, humic and fulvic acids (Table 2). Humin P was determined by a difference method and therefore the small changes in amounts found were not considered to be significant. Incubation of the soils for 9 mo caused a decrease in the amount of alkali-hydrolyzable P as compared to the original soil and an increase in the fulvic P in the control soil. Comparison of the grass and cellulose treatments with the control shows that the major net change in organic P fractions was an increase in the amount of fulvic P. Humic P was reduced and alkali hydrolyzable P remained unchanged. Addition of P without grass or cellulose did not change any organic P fractions as they were found to contain amounts similar to those of the control soil.

The types of changes in the bicarbonate-extractable inorganic- and organic-P fractions and microbial P are shown in Fig. 4 which depicts the changes in these fractions in one treatment, viz. the addition of cellulose plus N. Microbial P increased

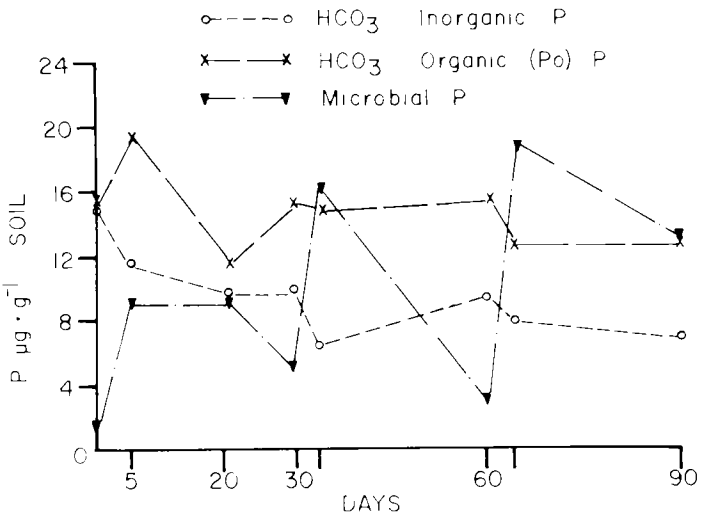

Fig. 4. Changes in $NaHCO_3$-extractable inorganic and organic P and in extractable microbial P in an Oxbow (Ap horizon) soil to which cellulose and N were added every 30 days.

rapidly at the beginning of each incubation period and when assessed again at the steady state conditions at the end of 30 days was found to be of a lower value. Inorganic P decreased during the initial build-up of a microbial population and in general remained constant at all other times at which it was measured. Bicarbonate-extractable organic P showed more fluctuation, decreasing slightly when the microbial P increased.

Concurrent microscopic measurements of bacterial and fungal biomass (Fig. 5) also showed fluctuations over the 30-day period with the maximum microbial biomass occurring 4–5 days following the addition of C. The points designated biomass A were calculated using normal literature values of 1.1 specific gravity and 80% H_2O as used by Babiuk and Paul (1970). The upper points (biomass B) were calculated using the conversion factors obtained from soil organisms grown at moisture tensions equivalent to those often found in soil. Van Veen and Paul (1979) have stated that they believe the factor for fungi which is 1.44 times the previously utilized values to be valid. However, they questioned their data concerning the high conversion factor for bacteria (3.63). The use of the higher values doubles the estimates for the average biomass from 304 to 663 $\mu g \cdot g^{-1}$ soil. The use of higher values has also increased the statistical significance between biomass and indices of microbial activity such as CO_2 evolution and ATP (Chauhan, Stewart and Paul, in preparation). The P immobilized during the first 4–5 days can be determined from the change in microbial P during this period. Table 3 shows that the increase in microbial P averaged 10.2 μg P $\cdot g^{-1}$ soil $\cdot mo^{-1}$ in the control treatments during the first 4–5 days during the initial 3 mo and 7.1 when averaged over 5 mo. The grass treatment showed an increase of 14.7 μg P $\cdot g^{-1}$ soil $\cdot mo^{-1}$ during the first 3 mo and 11.3 over the 5-mo period. Examination of the amounts of CO_2 produced showed that after 3 mo the cellulose-treated soils did not arrive at a steady state in the 30-day decomposition period. Hence, data for microbial P uptake and new biomass production should be more accurate in the first 3 mo.

DISCUSSION OF RESULTS

This experiment has documented the

changes in P forms that can occur at a rapid rate following the addition of organic matter to soil incubated under favorable conditions. Increases in biomass P during the first 4–5 days of each incubation period after organic residue addition were found to average 12 μg P $\cdot g^{-1} \cdot mo^{-1}$ (range 10.2 to 14.7 μg P) over the first 3 mo incubation period. The C:P ratio of the new soil biomass was approximately 20:1 (Table 3) indicating a high immobilization potential. The C:P ratio of the total biomass, calculated from the data in Fig. 5, was approximately 35:1 indicating the higher P content of the new growth that developed after each substrate addition. The microorganisms utilized soil P partly from the labile, inorganic P pool (resin-extractable). Measurement of $NaHCO_3$-

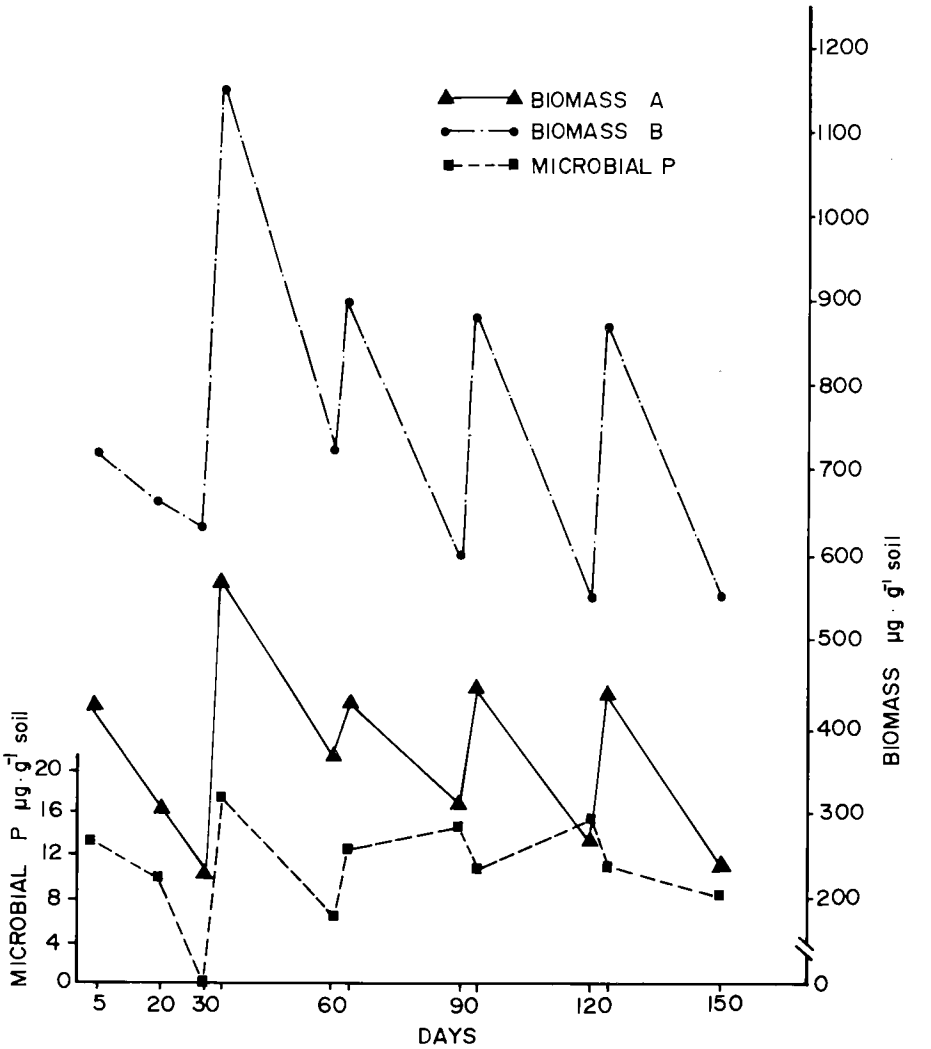

Fig. 5. Changes in biomass and microbial P in the cellulose plus N plus P treatment. Biomass A obtained by utilizing conversion values Babiuk and Paul (1970); biomass B for conversion factors used by van Veen and Paul (1979).

Table 3. Effect of treatment on the increase in microbial P and biomass observed in the first 4–5 days of each incubation period (data presented are the averages of the first three and five incubation periods)

	Change in microbial biomass averaged over		Change in microbial P averaged over		C:P ratio of new biomass	
	3 months	5 months	3 months	5 months	3 months	5 months
			$\mu g \cdot g^{-1}$ soil $\cdot mo^{-1}$			
Control	406	292	10.2	7.1	20:1	21:1
Grass + N	552	370	14.7	11.3	19:1	16:1
Cellulose + N	440	366	11.6	9.3	19:1	20:1
Cellulose + N + P	436	382	12.4	8.4	18:1	23:1
P	512	352	11.1	7.8	23:1	23:1

Grass and cellulose additions equivalent to 4000 kg $\cdot$ ha^{-1} every 30 days.

extractable organic P and total organic P also showed that considerable mineralization of the organic P occurred each month in treatments where net mineralization occurred. This agrees with measurements obtained by Cole et al. (1978) in microcosm studies. Monthly changes in biomass and P cycling were mainly attributable to C additions but partially affected by mixing as shown by the control.

In this experiment, the total uptake of P by microbial biomass during the first 4–5 days of each incubation period was affected only slightly by addition of organic residue N and fertilizer P during the first 3 mo incubation. Fractionation of both inorganic P and organic P at the end of 9 mo incubation showed that the addition of cellulose without fertilizer P depleted the inorganic phosphate pool in the soil by reducing the amount of NH_4F-extractable P and to a lesser extent NaOH-NaCl-extractable P. Presumably if the addition of cellulose without P had continued for a longer period of time the reserve of labile inorganic P pool (resin-P) would be seriously depleted. In the absence of resin-P the microbial population would be dependent on the rate of mineralization of organic P to supply P for uptake.

Although plants were not used in this study, immobilization of P by C addition and subsequent pool size depletion showed the possibility of gaining enough of an understanding of P redistribution to make a mathematical description of the processes possible. Results obtained for total P and total inorganic P showed greater precision than usually noted in fractionations such as this. This can be attributed to the care taken in preparation and mixing of soils prior to all subsampling. Recovery values of added P were excellent with the exception of the cellulose + P treatment which had a recovery value of 89% of added P. Later independent analysis of these samples for P by other sequential extraction techniques (as yet unpublished) have shown very good agreement between the replicate samples obtained from this incubation. Data obtained from two other soils using the same mixing, subsampling and incubation techniques also have been good. These show a range of recoveries from 90 to 111% which is slightly larger than the range of 89–100% obtained in this experiment. The fractionation techniques were reproducible and the movement of P between fractions was consistent with the known chemistry and biology of soil P. Although further refinements in techniques are necessary, the data presented indicate that the dynamics of P movement may be measurable with greater ease and accuracy than that of nitrogen and carbon.

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AMER, F., BOULDIN, D. R., BLACK, C. A. and DUKE, F. R. 1955. Characterization of soil phosphorus by anion exchange resin adsorption and P^{32} equilibration. Plant Soil **6**: 391–408.

ANDERSON, G. 1960. Factors affecting the estimation of phosphate-esters in soils. J. Sci. Food Agric. **11**: 497–503.

BABIUK, L. A. and PAUL, E. A. 1970. The use of fluorescein isothiocyanate in the determination of the bacterial biomass of a grassland soil. Can. J. Microbiol. **16**: 57–62.

BOWMAN, R. A. and COLE, C. V. 1978a. Transformations of organic phosphorus substrates in soils as evaluated by $NaHCO_3$ extraction. Soil Sci. **125**: 49–54.

BOWMAN, R. A. and COLE, C. V. 1978b. An exploratory method for fractionation of organic phosphorus from grassland soils. Soil Sci. **125**: 95–101.

BOWMAN, R. A. and COLE, C. V. 1979. Estimation of microbial P in soils using a $CHCl_3$ treatment technique. Soil Biol. Biochem. (in press).

COLE, C. V., ELLIOTT, E. T., HUNT, H. W. and COLEMAN, D. C. 1978. Trophic interactions in soils as they affect energy and nutrient dynamics. V. Phosphorus transformations in simulated rhizospheres. Microb. Ecol. **4**: 381–387.

COLE, C. V., INNIS, G. S. and STEWART, J. W. B. 1977. Simulation of phosphorus cycling in semiarid grasslands. Ecology **58**: 1–15.

COLEMAN, D. C., COLE, C. V., ANDERSON, R. V., BLAHA, M., CAMPION, M. K., CLARHOLM, M., ELLIOTT, E. T., HUNT, H. W., SHAEFER, B. and SINCLAIR, J. 1977. An analysis of rhizosphere-saprophage interactions in terrestrial ecosystems. *In* U. Lohn and T. Persson, eds. Proc. VI Colloquium of Soil Zoology. NFR Bull. No. 25, Uppsala, Sweden.

ELLIS, J. G., ACTON, D. F. and CLAYTON, J. S. 1965. The soils of the Regina map area. Ext. Publ. 176, University of Saskatchewan, Saskatoon, Sask.

HALM, B. J., STEWART, J. W. B. and HALSTEAD, R. L. 1972. The phosphorus cycling in a natural grassland ecosystem. Pages 571–586 *in* Isotopes and radiation in soil-plant relationships including forestry. I.A.E.A.,Vienna.

HANNAPEL, R. J., FULLER, W. H. and FOX, R. H. 1964. Phosphorus movement in a calcareous soil. II. Soil microbial activity and organic phosphorus movement. Soil Sci. **97**: 421–427.

OLSEN, S. R., COLE, C. V., WATANABE, F. S. and DEAN, L. A. 1954. Estimation of available phosphorus in soils by extraction with sodium bicarbonate. U.S. Dep. Agric. Circ. 939.

PAUL, E. A. and JOHNSON, R. L. 1977. Microscopic counting and ATP measurement in determining microbial growth in soils. Appl. Environ. Microbiol. **34**: 263–269.

PETERSON, G. W. and COREY, R. B. 1966. A modified Chang and Jackson procedure for routine fractionation of inorganic soil phosphate. Soil Sci. Soc. Amer. Proc. **30**: 563–565.

SADLER, J. M. and STEWART, J. W. B. 1975. Changes with time in form and availability of residual fertilizer phosphorus in a catenary sequence of Chernozemic soils. Can. J. Soil Sci. **55**: 149–159.

STEWART, J. W. B., HALM, B. J. and COLE, C. V. 1973. Nutrient cycling. I. Phosphorus. Tech. Rep. No. 40, International Biological Programme, Matador.

VAN VEEN, J. A. and PAUL, E. A. 1979. Conversion of biovolume measurements of soil organisms, grown under various moisture tensions, to biomass and their nutrient content. Appl. Environ. Microbiol. **37**: 686–692.