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LABILE SOIL PHOSPHORUS AS INFLUENCED BY METHODS OF APPLYING RADIOACTIVE PHOSPHORUS

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Abstract. The influence of different methods of applying radioactive phosphorus on the E- and L-values was studied in four foil types using barley, buckwheat, and rye grass for the L-value determination. The four soils differed greatly in their E- and L-values. The experiment was carried out both with and without carrier-P. The presence of carrier-P had no influence on the E-values, while carrier-P in some cases gave a lower L-value. Both E- and L-values depended on the method of application. When the ^{32}P was applied on a small soil or sand sample and dried before mixing with the total amount of soil, the E-values were higher than at direct application most likely because

(continued on next page)

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of a stronger fixation to the soil/sand particles. This was not the case for the L-values that are based on a much longer equilibrium time. On the contrary, the direct application of the ^{32}P-solution to the whole amount of soil gave higher L-values of a non-homogeneous distribution of the ^{32}P in the soil.

INIS Descriptors: ISOTOPIC EXCHANGE, PHOSPHORUS, PHOSPHORUS 32, PLANTS, AOILS.

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1. INTRODUCTION

Radioactive phosphorus is widely used by agricultural research workers in studies concerning the phosphorus nutrition of plants. Plant available or labile soil phosphorus has been measured by the isotopic dilution technique, both in laboratory and greenhouse experiments, and the results have been reported as E- and L-values, respectively. Some apparent discrepancies between conclusions drawn from evaluations of E- and L-values may be ascribed to differences in experimental procedures.

The E-value of a soil is commonly defined as the amounts of phosphorus on the surface of soil particles and in the soil solution that is exchangeable with orthophosphate ions added in solution. However, the extent to which soil phosphorus exchanges with added radioactive phosphorus (^{32}P) depends on the experimental conditions, e.g., the time allowed for isotopic equilibration. For E-value determinations, Amer (1962) equilibrated for ½ hour, Rennie and McKercher (1959) 1 hour, Mattingly and Talibudeen (1960) 20 and 170 hours, Russell et al. (1954) and Fried (1957) 48 hours, Olsen (1952) 50-70 hours, and Gunnarson and Frederiksson (1951) 14 days. The methods of applying the radioisotope to the soil and the soil-to-solution ratio used in the equilibration suspension influences the E-values obtained (Russell et al. 1954, Wiklander 1950, McAucliff et al. 1948, Talibudeen 1957, Ipinmidun 1973). Consequently, the E-values reported by different investigators may not be comparable unless the conditions for their determinations are identical. The method described in the "Tracer Manual on Crops and Soils" (IAEA-Techn Rep. Series 171, 1976) was used as the reference method in the present investigations on isotopic exchange in suspensions of various soils.

The L-value was defined by Fried (1964) as "the amount of phosphorus in the soil and in the soil solution that is exchangeable with orthophosphate ions added to the soil as measured by a plant growing in the system". This definition was agreed upon by Larsen (1967) but he stressed that "only when isotopic equilibrium is obtained does the L-value signify a definite quantity of soil phosphorus". If isotopic equilibrium is established between the ^{32}P added and a labile fraction of phosphorus in the soil, the L-value should be independent of both crop and the growing period. Lack of isotopic equilibrium may be a reason for conflicting results reported from different experiments. Another cause may be that plant roots affect the solubility of soil phosphorus by differing amounts.

The present investigations were carried out in order to evaluate if the method of application of ^{32}P affected the extent of isotopic exchange in different soil types. Furthermore, it was investigated if the previously observed differences among plant species (Andersen and Thomsen 1978) depended on soil characteristics.

2. MATERIALS AND METHODS

Some characteristics of the four soils used in both the laboratory- and the pot experiment are given in Table 1. Soil no. I and II were collected from the experimental field at Risø while no. III and IV were taken from different plots of the long term fertilizer experiment at the Agricultural University, Copenhagen.

2.1. Laboratory experiment (E-values)

Radioactive phosphorous was added to soil samples in four different ways as described below. Each sample was supplied with 7 μCi ^{32}P carrier-free or at a specific activity of 1.22 μCi/mg P.

Table 1. Characteristics of the experimental soils

Soil no.	I	II	III	IV
Soil properties	Sandy loam	Sandy clay loam	P-deficient clay loam	Clay loam
pH (H_2O)	5.6	8.0	6.6	5.4
Clay (%)	4.4	12.3	20.0	21.0
Organic matter (%)	1.6	2.0	2.0	1.8
C.E.C. (meq/100 g)	6.9	19.0	20.0	20.1
Base saturation (%)	43	87	80	59
Total inorganic P mg/g soil	250	110	270	300

The sand used in these experiments is acid-washed quarts sand

a. Five gram soil samples were shaken with 49 ml 0.01 M $CaCl_2$ for 24 hours before addition of ^{32}P and a further 48 hours shaking (E-value as described in IAEA manual Technical Report Series 171).
b. ^{32}P was added to 5 g soil samples and shaken with 5 g sand in 49 ml 0.01 M $CaCl_2$ for 48 hours.
c. ^{32}P was added to 5 g soil samples, air-dried and shaken with 5 g sand in 50 ml 0.01 M $CaCl_2$ for 48 hours.
d. ^{32}P was added to 5 g sand, air-dried and shaken with 5 g soil samples in 50 ml 0.01 M $CaCl_2$ for 48 hours.

All treatments were carried out with four replicates.

The suspensions were centrifuged, and the radioactivity of the supernatents was measured by liquid scintillation counting (without the use of a scintillator). The total phosphorous content of the supernatents was determined by the ascorbic acid method (John, 1969). The isotopic exchangeable P was calculated using the formula

$$E_{48} = \frac{r_i}{r_f} \times {}^{31}P_f \ \mu g \ P/g \text{ soil}$$

where r_i and r_f are the count rates of the initial and final ^{32}P solutions, respectively, and $^{31}P_f$ is µg P in the final solution.

2.2. Pot experiment (L-value)

Experimental procedure. The L-value experiment was carried out with three test crops: Rye grass (Lolium perenne, var. DUX Øtofte), barley (Hordeum vulgare, var. Lofa), and buckwheat (Fagopyrum esculentum). The plants were grown in 2-kg pots in a 1:1 mixture of one of the four soils and sand. ^{32}P was added to the soil mixtures at 75 µCi/pot carrier-free or with a specific activity of 1.22 µCi/mg P as NaH_2PO_4. Three methods of application were used:

L. The ^{32}P-solution was added to the surface of 1 kg of the soil sand mixture, covered with another kg and mixed further into 22 kg.

M. The ^{32}P-solution was added to 50 g soil, air-dried and mixed into a total of 24 kg of soil mixture.

N. The ^{32}P-solution was added to 50 g sand, air-dried and mixed into a total of 24 kg of soil mixture.

Each treatment was carried out in 4 replicates. One hundred seeds of rye grass, 5 seeds each of barley and buckwheat were sown in each pot on 11 April 1979. The seed phosphorus in the above number of seeds used were 0.66, 0.64 and 0.36 mg respectively. The pots were placed in a greenhouse randomized within three groups, each comprising a single crop.

NH_4NO_3 (200 mg N/kg soil) was given twice to barley and buckwheat (6 and 26 days after sowing) and 3 times to rye grass (6, 26, and 42 days after sowing). Barley and buckwheat were harvested 63 days after sowing (12 June 1979). Rye grass were cut 42 and 64 days after sowing (22 May and 13 June 1979).

Preparation of plant material. The plant material was dried at 80°C for 24 hours and the dry matter yield was determined.

A dry ashing technique was used to bring the ground plant material into solution. 1.5 g plant powder was pressed into briquettes, dried at 80°C for 5 hours, weighed and combusted overnight. 10 ml of 1N HCl was added to the ash and an aliquote of the solution was used for determination of ^{32}P by liquid scintillation counting (without the use of a scintillator). Total phosphorus content was determined by the vanadomolybdate method as described by Benjaminsen & Jensen (1955).

Calculation of L-value. The L-value of a soil is defined as the amount of phosphorus in the soil and soil solution that is exchangeable with orthophosphate ions added to the soil and measured by plant uptake from the soil system (Fried, 1964). The amount of labile soil phosphate (L-value) can be calculated as follows:

$$L = \frac{^{32}P \text{ added to soil} \times (^{31}P \text{ in plants} - 2/3^{rd} \text{ seed } ^{31}P)}{^{32}P \text{ taken up by the plants}} - {}^{31}P \text{ added as carrier}$$

3. RESULTS AND DISCUSSION

3.1. E-value

Table 2 shows the amount of ^{32}P extracted from the soil samples in per cent of that added to the soils, and the amount of ^{31}P extracted per g soil. It is seen that the soil type, method of P-application, as well as the presence or absence of carrier-P all have a strong inpact on the extractability of ^{32}P and ^{31}P. In Table 3 the E-values for the four soils as affected by method of application and presence of carrier are given.

a. E-values of different soil types

When comparing the E-values with the total inorganic phosphorus of the four soils a clear relation is seen. The sandy clay loam with 110 µg inorganic P/g soil gives the lowest E-values in all cases, while the clay loam with 300 µg P/g soil has E-

Table 2. ^{32}P and stable P extracted from four soils with 0.01 M $CaCl_2$ as affected by method of ^{32}P application. Means of four replicates

Recovered P	^{32}P, % of added								µg ^{31}P/g soil (ppm)							
^{32}P added	carrier-free				with carrier				carrier-free				with carrier			
method / soil no.	a	b	c	d	a	b	c	d	a	b	c	d	a	b	c	d
I	4.44	2.87	2.90	2.00	47.6	35.0	18.0	26.8	2.1	1.2	1.8	1.8	169	122	68	100
II	2.54	1.77	1.61	1.09	54.3	45.1	25.8	33.7	0.68	0.20	0.74	0.58	181	151	92	121
III	2.00	1.33	1.13	1.00	45.5	39.7	20.8	30.4	1.0	0.39	0.69	0.72	160	137	77	123
IV	1.29	0.82	0.84	0.60	25.6	23.4	8.67	18.8	1.1	0.43	0.94	1.02	97	89	43	87

Table 3. Isotopically exchangeable P (E-values) of four soils as affected by the method of ^{32}P application. Means of four replicates.

Soil no.	Total inorganic P, μg P/g soil	E-value, g P per g soil							
		carrier-free				with carrier			
		Method of ^{32}P application							
		a	b	c	d	a	b	c	d
I	250	46	40	62	87	44	44	71	66
II	110	27	11	45	54	25	23	53	54
III	270	51	29	61	72	46	39	66	92
IV	300	82	52	111	169	69	70	180	150
c.v. %		12	27	11	27	21	8	44	19

values from 2 to 4.5 times higher. Therefore, the E-values relative to the total content of inorganic soil-P is given in Figure 1, and these results show the lowest relative E-values for the P-depleated clay loam.

b. Effect of carrier-P

The mean E-values over soils and methods of application are 63 and 59 μg P/g soil for carrier-free application of ^{32}P and carrier application, respectively. Considering the variation in the results, the difference between these figures is not statistical significant, which is in accordance with the theory of the E-value.

c. Effect of method of ^{32}P application

There is a great influence on E-values from the method of application of phosphorus. Methods c and d that both include air-drying of the soil and sand after P-addition give very high E-values; this might be explained by a fixation of the ^{32}P to an extent such that only part of it is equilibrated with the soil-P during the extraction period. This theory is confirmed by the results in Table 2 that show a greater extraction of

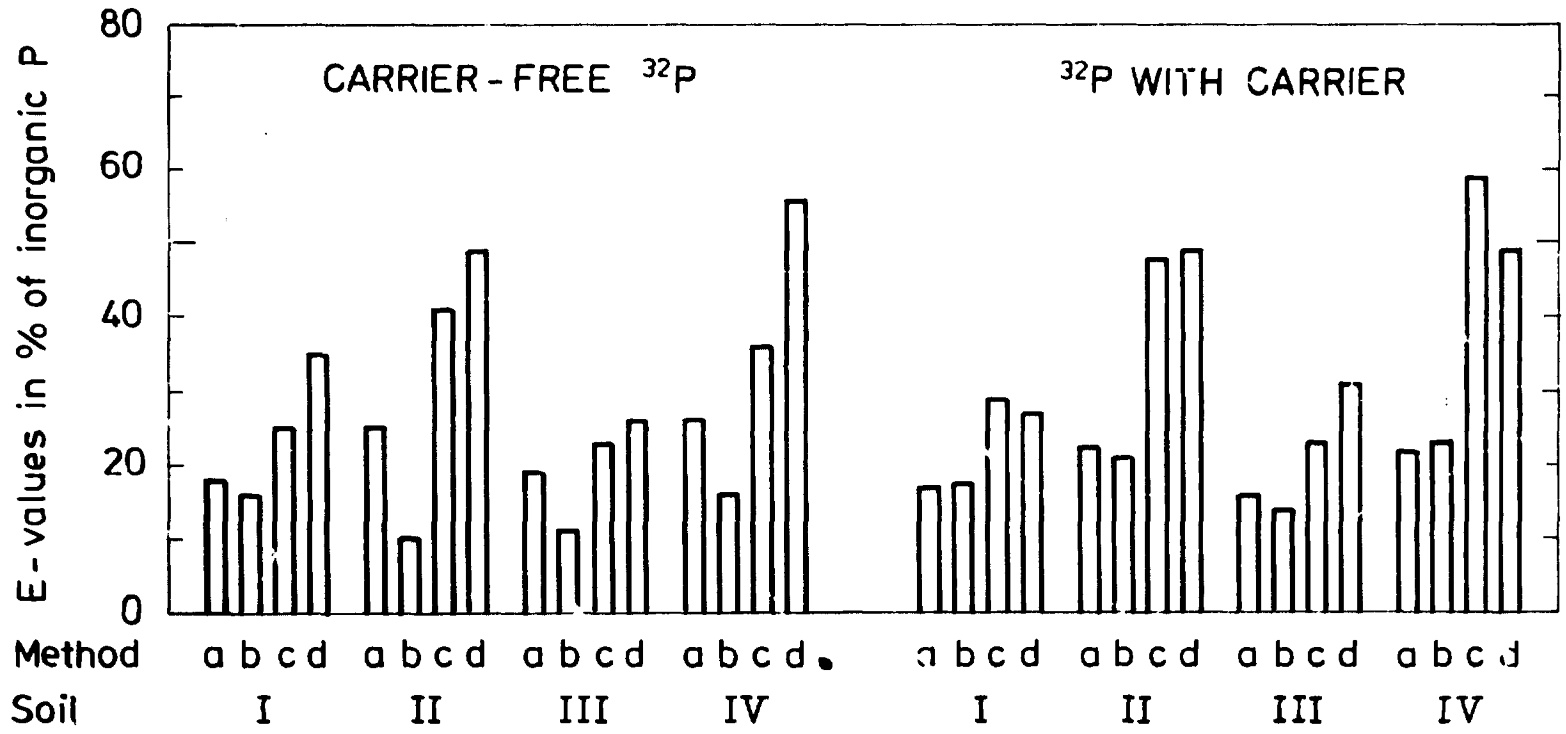

Figure 1. E-values relative to total inorganic P content of the four soils.

^{32}P at methods a and b than c and d, both with and without the carrier.

3.2. L-value

Dry matter yield and phosphorus concentration of ryegrass, barley and buckwheat grown on the four soils are given in Tables 4 and 5, respectively. The values are means of the four replicates and the three methods of ^{32}P application, since no significant difference was observed in the above parameters among the methods of application.

Table 4. Dry matter yield (g pot) of ryegrass, barley, and buckwheat grown in four soils. Means of four replicates and three methods of ^{32}P application

^{32}P added	carrier-free				with carrier			
Crop / Soil no.	Rye grass cut 1	Rye grass cut 2	Barley	Buck-wheat	Rye grass cut 1	Rye grass cut 2	Barley	Buck-wheat
I	3.28	3.82	5.60	6.10	5.79	5.45	11.9	17.4
II	3.47	3.41	3.10	10.4	6.71	5.52	14.2	24.3
III	4.19	3.88	5.90	13.2	5.64	5.42	7.80	14.4
IV	4.72	4.83	8.20	13.7	4.58	4.79	12.7	16.2
c.v.(%)	16.5	15.4	26.7	49.1	10.5	16.2	21.9	26.9

Table 5. Phosphorus concentrations (mg P/g d.m.) of ryegrass, barley, and buckwheat grown in four soils. Means of four replicates and three methods of ^{32}P application.

^{32}P added	carrier-free				with carrier			
Crop / Soil no.	Rye grass cut 1	Rye grass cut 2	Barley	Buck-wheat	Rye grass cut 1	Rye grass cut 2	Barley	Buck-wheat
I	2.46	2.05	2.04	1.37	3.69	3.01	2.49	2.20
II	1.84	2.10	1.58	1.31	3.86	2.94	2.10	2.34
III	3.81	3.43	1.80	2.45	4.91	3.98	2.55	4.04
IV	3.83	3.55	1.75	3.26	5.16	4.52	2.44	3.67
c.v.(%)	8.2	14.4	12.6	31.9	6.0	11.2	13.9	24.7

The results in Table 4 and 5 show that dry matter production and phosphorus concentration differed widely among the four soils and among the three plant species. Both the yield and the phosphorus concentration increased significantly with addition of carrier-P for all four soils, indicating that they all had too little labile phosphorus to meet the requirement of the plants.

Labile phosphorus (L-value) of the four soils as affected by the methods of ^{32}P application with and without the carrier is given in Table 6. The values are the means of four replicates. The L-values of the four soils relative to the total inorganic P of the soils are presented in Figure 2.

a. L-values of different soil types

It is evident from Table 6 that L-values of the four soils vary according to their total inorganic P content, showing highest values in the clay loam soil. The sandy clay loam with a total inorganic P content of 110 mg P/kg soil, gives the lowest L-value in all the three test crops with and without application of carrier-P. When considering L-values relative to the total inorganic P, the values are higher for the sandy clay loam than those from the sandy loam, since the sandy loam has twice as much total inorganic P (250 mg/kg soil) as the sandy clay loam. The L-values relative to the total inorganic P give therefore a more comparable picture of the availability of the soil phosphorus than the L-values itself. Andersen et al. (1961) found in the investigation of L-values with different soil types that soils vary in the L-values according to their total inorganic P content.

b. Effect of plant species

The variation in L-values among plant species is evident from Table 6, barley giving the lowest L-values in most cases. However, an interaction is seen between plant species and soil types; thus the L-values are the same for barley and buckwheat grown on soil no. I. Russell et al. (1958) report the differ-

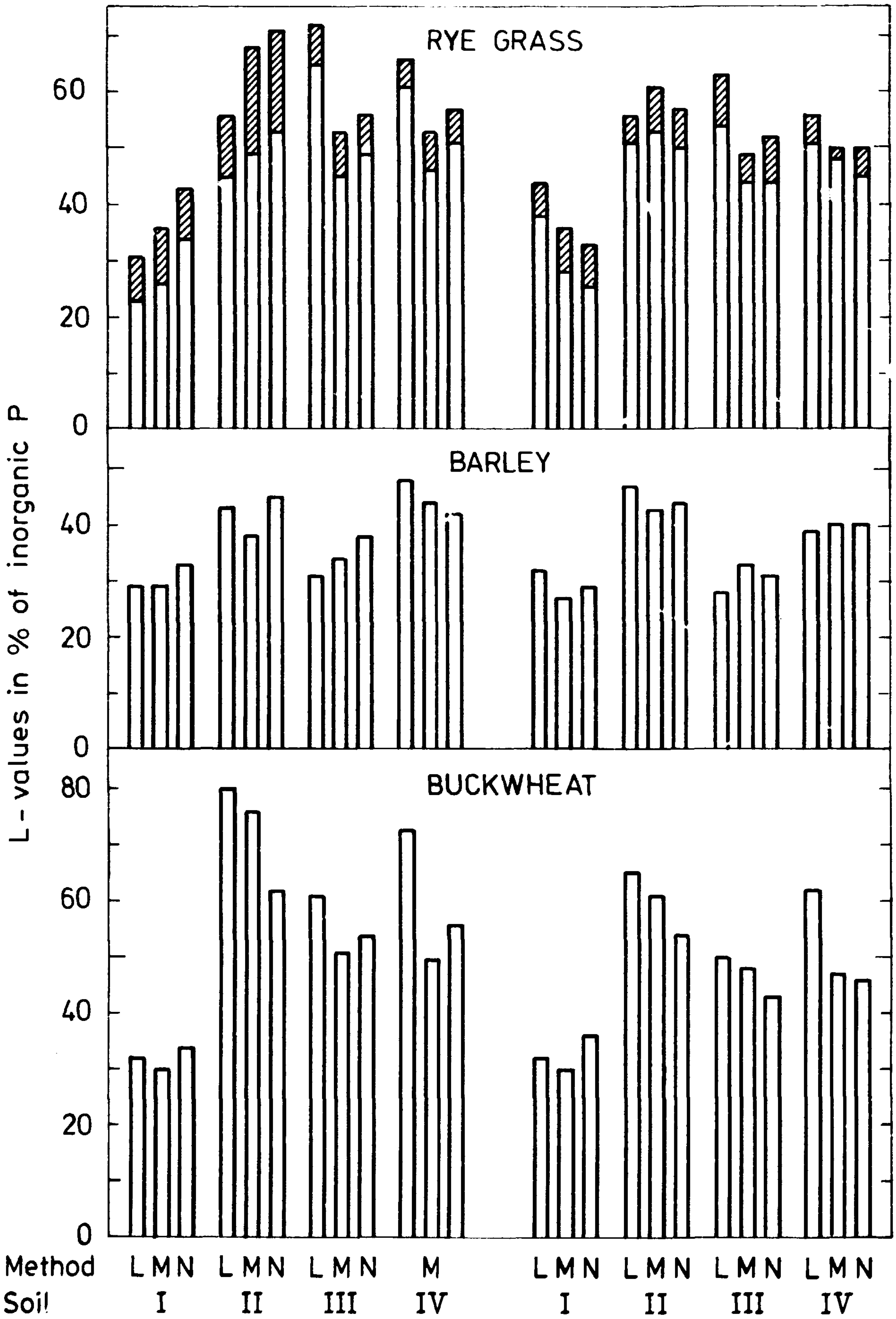

Figure 2. L-values of the crops relative to total inorganic P content of the four soils. The shaded areas represent the increase of L-values from first to second cut.

Table 6. Labile phosphorus (L-value, mg P/kg soil) of four solids as affected by the method of ^{32}P application with and without carrier. Means of four replicates

carrier-free ^{32}P	Rye grass cut 1			Rye grass cut 2			Barley			Buckwheat		
Method of appl.* / Soil no.	L	M	N	L	M	N	L	M	N	L	M	N
I	58	66	84	78	90	108	73	72	83	79	75	86
II	50	54	58	62	75	78	47	42	50	88	84	68
III	176	121	130	195	144	150	84	91	102	164	139	146
IV	184	139	153	199	159	172	145	131	125	218	149	167
c.v.(%)	48	4	5	45	8	9	61	10	13	38	9	11

^{32}P with carrier	Rye grass cut 1			Rye grass cut 2			Barley			Buckwheat		
Method of appl.* / Soil no.	L	M	N	L	M	N	L	M	N	L	M	N
I	96	70	66	109	90	83	79	67	72	81	74	66
II	56	58	55	62	67	63	53	47	48	71	68	59
III	147	120	120	171	132	137	75	88	83	134	126	116
IV	153	144	136	167	150	151	116	121	120	187	142	137
c.v.(%)	16	10	9	19	9	5	19	7	7	16	8	12

* Method L, M, and N: see materials and methods

ences among plant species are believed to reflect the ability to lower the free energy of phosphate in the external medium, and they state that barley is reputedly of low efficiency in absorbing phosphate from acid soils. Of the three plant species buckwheat gave the highest L-values in most soils. This is in accordance with Russell (1973) and Larsen (1973) who described buckwheat as a very P-efficient crop.

The ph values of rhizosphere soils measured after cropping of the four soils are given in Table 7. It is evident from this

Table 7. $pH_{(0.01\ M\ CaCl_2)}$ of rhizosphere soil measured after cropping the soils

Soil no.	Control	Rye grass	Barley	Buckwheat
I	7.50	7.47	7.89	7.43
II	8.00	6.10	6.45	5.10
III	6.60	5.30	5.74	4.85
IV	5.40	4.92	5.49	4.55

table that plants vary in their ability to change the pH of the soils in which they were grown. Buckwheat seems to decrease the pH of soils markedly, while barley in two of the soils increased the pH. It is evident that the pH was reduced more in the two clay loams (no. III and IV) and the sandy clay loam (no. II) than in the sandy loam (no. I). Buckwheat is a crop showing an alkaline uptake, which is defined as the property to absorb more cations than anions. Due to its highly alkaline uptake, buckwheat exerts a strongly acidifying effect on its soil environment (Van Ray, 1979). It is clear that buckwheat exerts a strong acidifying effect on the soils in which it is grown in the present study except in sandy loam.

Figure 2 indicates that L-values relative to total inorganic P content of the four soils in which buckwheat was grown shows higher relative values than those in ryegrass and barley; buckwheat is thus seen to be an efficient crop in P utilization.

c. Effect of carrier-P

The use of carrier-P tended to give lower L-value results in the two clay loams (nos. III and IV), but no clear trend was found in the other two soils. The most significant influence of carrier-P is the considerable decrease in the coefficient of variation (c.v.) for method L, indicating a much more homogeneous distribution in the soil of the ^{32}P when carrier-P is added along with the ^{32}P.

d. Effect of method of ^{32}P application

The L-values of the four experimental soils differ widely depending on the method of ^{32}P application (Table 6). In most cases the L-values were higher for method L (surface application) than for methods M and N, while there was no difference whether the ^{32}P was added to a subsample of soil (method M) or sand (method N) before mixing with the rest of the soil. This was seen both with and without carrier application.

One cannot conclude from these results which of the two methods M or N is most efficient in regard to different plant species. The coefficients of variation of the L-values over the four soils were higher at surface application of ^{32}P (method L) than at the other two methods, which must be due to a nonhomogeneous distribution. When ^{32}P is applied to the soil surface, part of the activity might be fixed to the soil in small lumps that are difficult to distribute evenly into a large volume of soil. This results in the higher coefficient of variation seen in Table 6. Furthermore, an interaction is observed between the method of application and soil type and plant species. This implies that discrepancies in the literature about L-values related to plant species might to some extent be a result of the use of different methods of application of the ^{32}P.

For this reason the surface method of ^{32}P application should be avoided in research work. Furthermore, the greater variation between replicates in this method might cover variations due to other factors under consideration.

4. CONCLUSION

No concepted differences exist between the E- and L-value; only the nature of extractants and time of extraction differ, the E-value being based on liquid extractions in a few days and L-value using plants to extract the phosphorus during weeks or months. However, this difference has a great impact on the suitability of different methods of ^{32}P-application. Air-drying of the P-solution placed on sand or soil samples gives a homogeneous distribution which is an advantage in the case of L-value determination, but it also gives a great initial fixation; this fixation is, however, a disadvantage for the E-value determination, while it is of minor importance in case of L-value determination because of the longer period of equilibrium.

Further, the interaction observed between soil type, method of ^{32}P-application and plant species on the L-values indicates the importance of using not only the same plant species but also the same method of ^{32}P-application when the L-values of different soils are compared.

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