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THE GROWTH RESPONSES OF *PROTEA CYNAROIDES* L. TO DIFFERENT LEVELS OF PHOSPHORUS AND NITROGEN

ECOLOGY PROJECT

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Botany Honours
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C O N T E N T S

ABSTRACT

The study was designed to observe the growth response and the response of the lignotuber of Protea cynaroides L. to varying levels of P and N. Seeds were germinated and fed once weekly on a modified Hoagland's solution diluted to give constant ratio and varying ratio concentrations of P and N. Growth was inhibited by high concentrations of P and N in the ratio 1 : 4 ppm but not by high concentrations in a different ratio. The results are discussed in terms of previous studies and in terms of uncontrolled variables. Suggestions for improvement in this type of study are made.

INTRODUCTION

The remarkable convergence in physiognomy in the vegetation of the mediterranean-type climatic areas of the world has been the subject of intense interest and speculation for some time. Much emphasis has been placed on descriptions and comparisons of the common life-forms constituting the evergreen sclerophyllous vegetation of these areas and on relating these forms to similar climatic conditions (e.g., Naveh, 1967 and Specht, 1969).

Of particular interest is the presence of typical heathland vegetation in the mediterranean-type climatic areas of the South-western Cape and South and Western Australia, but not in the other mediterranean areas, i.e., Chile, California and the Mediterranean basin. In Australia, the eco-physiology of the heathland vegetation has been the particular interest of workers like Beadle (1954, 1966, 1968) and Specht (1963, 1969, 1973). Specht and Rayson (1957) have, for instance, reported that although the climatic conditions under which the heath on their study site on Dark Island grows, may vary considerably, the soils on which it flourishes are always acid and always low in available phosphorous (P) and nitrogen (N) and occasionally in potassium, copper, zinc and mdybdenum. The important question has therefore been raised of how the sclerophyllous

vegetation survives on soils of such low nutrient status. This is a question which is still a long way from being answered satisfactorily although a number of mechanisms have been elucidated (e.g., the ability of heathland plants to store organically bound phosphates in the roots and to hydrolyse and release them during late spring - early summer (Specht and Groves, 1966).

In the Cape the fynbos vegetation is subject to the same types of environmental restraints which are found in Australian heathlands, i.e. summer droughts and soils low in essential nutrients. A full-scale programme to investigate the physiology of plants surviving in these conditions has only very recently commenced and a primary aspect of the programme is to investigate the dynamics of plant mineral nutrition on Cape dystrophic soils.

The present project was suggested with this background in mind and was aimed essentially at observing the growth response of a well-known fynbos shrub Protea cynaroides L. to different soil concentrations of P and N. It was hypothesised that because of an adaption to low nutrient fynbos soils the plant would not respond positively to very high concentrations of P and N. The project is also in the way of a pilot study, looking at some of the practical methodological problems involved with a controlled

autecological study of this sort.

The reason for choosing Protea cynaroides as the study specimen brings us to a secondary purpose of this project. This was to observe whether soil mineral status is an important factor in the growth and activity of the plant lignotuber. A lignotuber has been described by Mullette (1978) as "a swollen and partly buried portion of the main stem containing numerous dormant buds and food reserves". Lignotubers have been described for most species of Eucalyptus in Australia and Mullette (1978), Mullette and Bamber (1978) and Bamber and Mullette (1978) have investigated the anatomy and chemical composition of the lignotuber of Eucalyptus gummifera. The anatomy of the lignotuber suggests that it may contain a reserve of carbohydrates and chemical analyses indicated that it is an important storage organ for mineral nutrients. In relation to mineral nutrition it is of interest to ascertain whether the development of lignotubers is determined by the nutrient status of the soil or whether their development and activity is genetically determined. This would enable one to decide whether lignotubers represent a life-strategy selected by plants indigenous to the stressful heathland conditions found in mediterranean-type climatic areas. Beadle (1968) has investigated the effects of manipulating P and N soil levels on the growth of lignotubers of a number

of Eucalyptus species. He concluded that although nutrient supply does not alter the potential of a plant to produce lignotubers, limiting P appears to stimulate their increase in size. Mullette and Bamber (1978) found that though the presence of lignotubers is inherited their development is enhanced by greater soil P concentrations.

Although the storage function of lignotubers is still open to speculation, their regenerative function is not. After bushfires, new growth has been observed to develop from the lignotuber region which because of its partly buried situation is able to escape the worst effects of a fire. In mountain fynbos Protea cynaroides is a plant which prominently regenerates from its lignotuber after fire. Figure 1 demonstrates this regeneration in a P. cynaroides plant on the slopes of Happy Valley, Bain's Kloof, where a fire swept the area some 1½ years ago.

Lignotubers may therefore form an important survival strategy in vegetation indigenous to oligotrophic or dystrophic soils and subject to periodic fires, and their development and function in relation to edaphic conditions is of the utmost interest.

Figure 1

Protea cynaroides regenerating after fire from partly buried lignotuber. Photographed at Happy Valley, Bain's Kloof.

MATERIALS AND METHODS

Fresh seeds of P. cynaroides collected in Kirstenbosch Botanic Gardens were used. The seeds were sown in seed trays at a shallow depth in washed, autoclaved vermiculite of a pH of 6,5. The seed trays, sterilized with a 2,5% solution of sodium hypochlorite were kept in continual darkness in an incubation room whose temperature was maintained at 25°C. These conditions were selected in accordance with the findings of Horn (1962), van Staden (1966) and Brown and van Staden (1971).

The vermiculite was kept continually moist by watering with deionized water. First germination occurred after three weeks. Upon emergence of the cotyledons, the seedlings were transferred to individual plastic pots containing washed, autoclaved vermiculite. The pots were placed in a growth room under cool white 72" "Powertube" fluorescent lights giving a light intensity of 15 000 lux. Half-way through the experiment the lights were changed to 1 000 W "Philips" mercury vapour lamps supplemented with incandescent lamps. The light intensity varied from 11 000 lux. to 16 000 lux. and the temperature from 24°C to 28°C, depending upon the distance the plants were from the lamps. A 16-hour light regime was maintained throughout the course of the experiment.

The experiment design was as follows. P levels ranging from 50 ppm to 0,01 ppm were chosen to represent a sufficiently large range in concentrations. Nitrogen levels were adjusted in order to maintain a constant ratio between N and P. Thus treatments are hereafter referred to as $P_{50}N_{200}$, $P_{10}N_{40}$, P_1N_4, $P_{0,1}N_{0,4}$ and $P_{0,01}N_{0,04}$. N was supplied solely in the form of nitrates. For three of the treatments, i.e. $P_{50}N_{200}$, P_1N_4 and $P_{0,1}N_{0,4}$, control treatments in which N was kept constant at 40 ppm were included. This was to ascertain to what extent the ratio between N and P could be responsible for any observed differences in growth rates. A control treatment of seedlings growing in a shale-sandstone mixture and watered with deionized water was also included for comparative purposes.

Two days after transplanting, each plant was given 150 ml of a modified Hoagland solution of the appropriate dilution (Table 1). The concentrations of the major cations and the anions besides PO_4^{3-} and NO_3^- were kept constant for all treatments although in the case of Ca^{2+} and Na^+ this was not possible as their concentrations followed that of the nitrates. After the first week 100 ml of the feeding solutions were applied at weekly intervals. In between each feeding the vermiculite was washed through with deionized water in order to leach away salts residual from the previous feeding. The pH of all the treatment

TABLE 1 : COMPOSITION OF NUTRIENT SOLUTIONS

STOCK SOLUTIONS	
Basal Nutrients	Mass of salt per litre, individual stock solution
$Ca(NO_3)_2 \cdot 4H_2O$	135,00g
$NaNO_3$	24,28g
$NaH_2PO_4 \cdot 2H_2O$	25,16g
K_2SO_4	75,00g
$MgSO_4 \cdot 7H_2O$	24,77g
$CaCl_2 \cdot 6H_2O$	42,50g
Fe EDTA	
Micronutrients	

FEEDING SOLUTIONS				
Treatments	Concentration (ppm) of major cations per litre feeding solution+			
	Ca	Na	K	Mg
$P_{50}N_{200}$	353	103	168	25
$P_{50}N_{40}$	169	50	168	25
$P_{10}N_{40}$	169	21	168	25
$P_1 N_4$	124	2	168	25
$P_1 N_{40}$	169	14	168	25
$P_{0,1}N_{0,4}$	123	0,2	168	25
$P_{0,1}N_{40}$	169	13	168	25
$P_{0,01}N_{0,04}$	123	0,02	168	25

+ Feeding solutions for each treatment consisted of 10ml of each macronutrient stock solution, 1 ml of Fe EDTA stock solution, 1 ml from micronutrient stock solution, P and N stock of the appropriate dilution - all made up to 1 litre volume with deionised water.

solutions remained constant at 5,5 throughout the experiment. Each treatment aimed at a sample size of 5 replicates although this number diminished to 2 in some treatments owing to high mortality rates. The seedlings were harvested and measurements taken when they were all 14 weeks old.

RESULTS

At the outset it should be noted that an unexpected variable was discovered after some weeks' growth. The batch of seeds sown was found to have contained two variants of P. cynaroides. The one variant was broader-leafed with much shorter internodes, generally longer hypocotyls and altogether healthier and sturdier looking than the other variant which was thinner-leaved with longer internodes. Figure 2 (overleaf) demonstrates the differences between the two variants. It was not possible to discard either of the variants because of the already small sample sizes and consequently the mean values represented in the following histograms embody measurements of both variants.

Because of this variable it was not possible to make any assumptions with regard to the normalcy of the distribution of the population from which the sample seeds were randomly chosen. This factor together with the very small sample

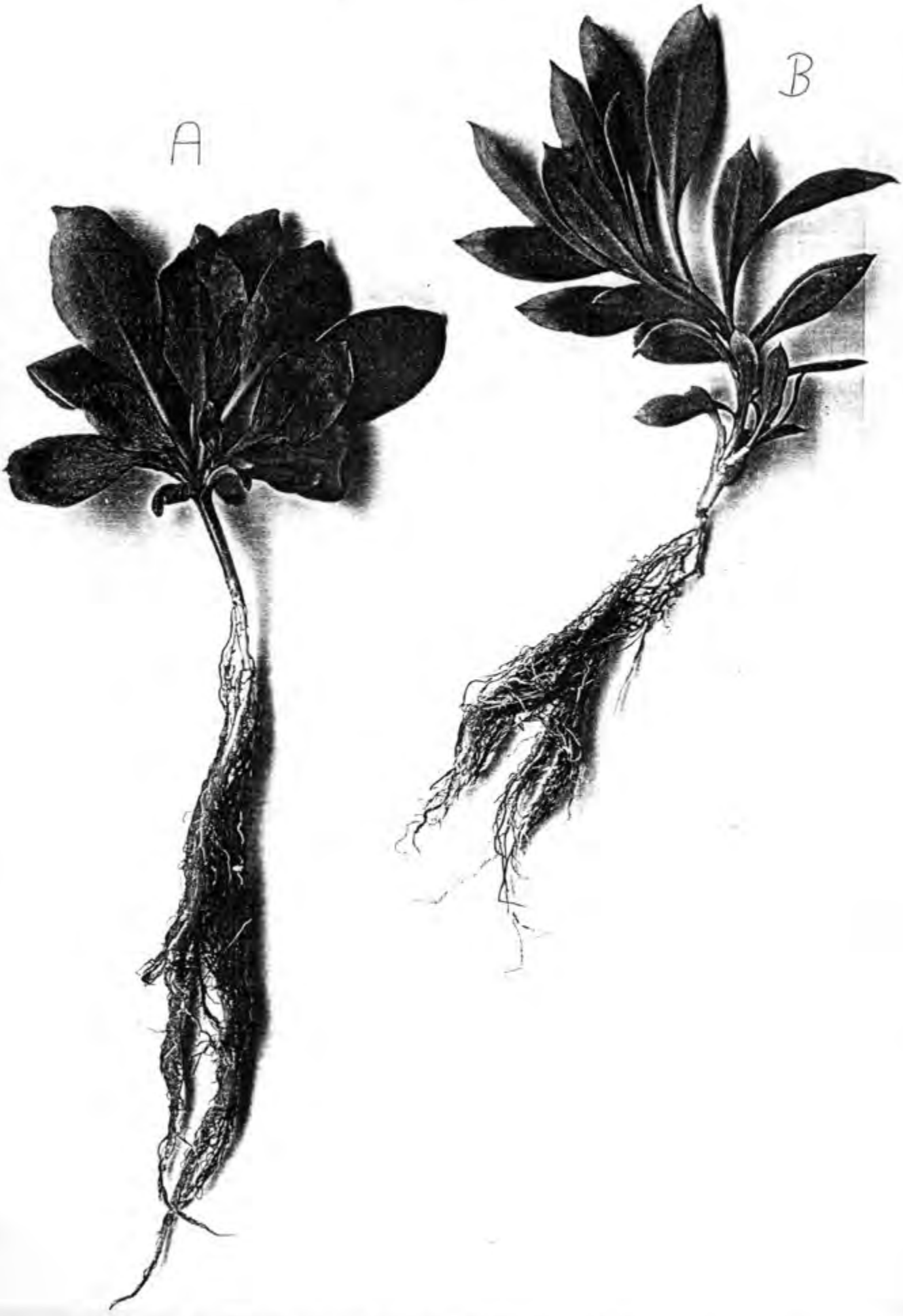

Figure 2 : Two Variants of Protea cynaroides :
A - Broad-leaved Variant; B - Narrow-leaved Variant.

sizes made it unsuitable to perform any statistical tests of significance on the data obtained. Standard deviations were calculated and have been shown where possible. Very large standard deviations resulting from large ranges in data within the small samples have not been indicated.

Referring to the constant ratio treatments only, i.e. $P_{0,01}N_{0,04}$, $P_{0,1}N_{0,4}$, P_1N_4, $P_{10}N_{40}$ and $P_{50}N_{200}$, and looking initially at fresh weight values Figures 5, 6 and 7 all show very similar trends, i.e. greatest growth occurring at the lowest level, $P_{0,01}N_{0,04}$, and lowest growth occurring at the highest treatment level, $P_{50}N_{200}$. Fresh weight is, however, a notoriously unreliable parameter, varying with relative humidity, time after watering, etc. and dry weight gives a far more reliable indication of relative biomass. Another reason for looking at dry weights is that on the day of harvesting the plants of the $P_{50}N_{200}$ treatment were found to have inexplicably wilted overnight. The fresh weight value presented for this group is therefore not true.

The dry weight values of the root system (Fig. 5), the aerial parts (Fig. 6) and the total dry weight biomass (Fig. 7) indicate a definite and constant gradation from highest values at the lowest nutrient treatment to lowest values at the highest nutrient treatment. The fact that the component parts of the plants follow the same trend

as the total biomass suggests that the effects of the different nutrient levels were felt by the plant as a whole.

Fig. 3 shows that the lignotubers follow the same growth trends as the other parts of the plant. The dip at the $P_{0,1}N_{0,4}$ treatment cannot be explained and may have been due to experimental error. Although this trend tends to suggest that lignotuber growth is modified by mineral environment in the way that Beadle (1968) has shown in Eucalyptus species, it should be remembered that at the time of harvesting the lignotubers were no more than a swelling with little observable difference between them and the stem and root regions above and below (Fig. 4). One cannot predict to what proportions they would have grown once the plant had passed the seedling stage. The lignotuber actually developed in the epicotyl region at the bases of and between the cotyledons. Despite the fact that lignotuber biomass was lowest at the $P_{50}N_{200}$ level, there were very noticeable differences in their activity at this level. Whereas the soil plants had sprouted one or two small leaves from the lignotuber region and the lower P and N treatments had sprouted between 2 and 5 small- to medium-sized leaves and at the most 2 small shoots, the $P_{50}N_{200}$ treatments sprouted between 12 and 15 small to medium leaves. The $P_{50}N_{200}$ plants sprouted, in

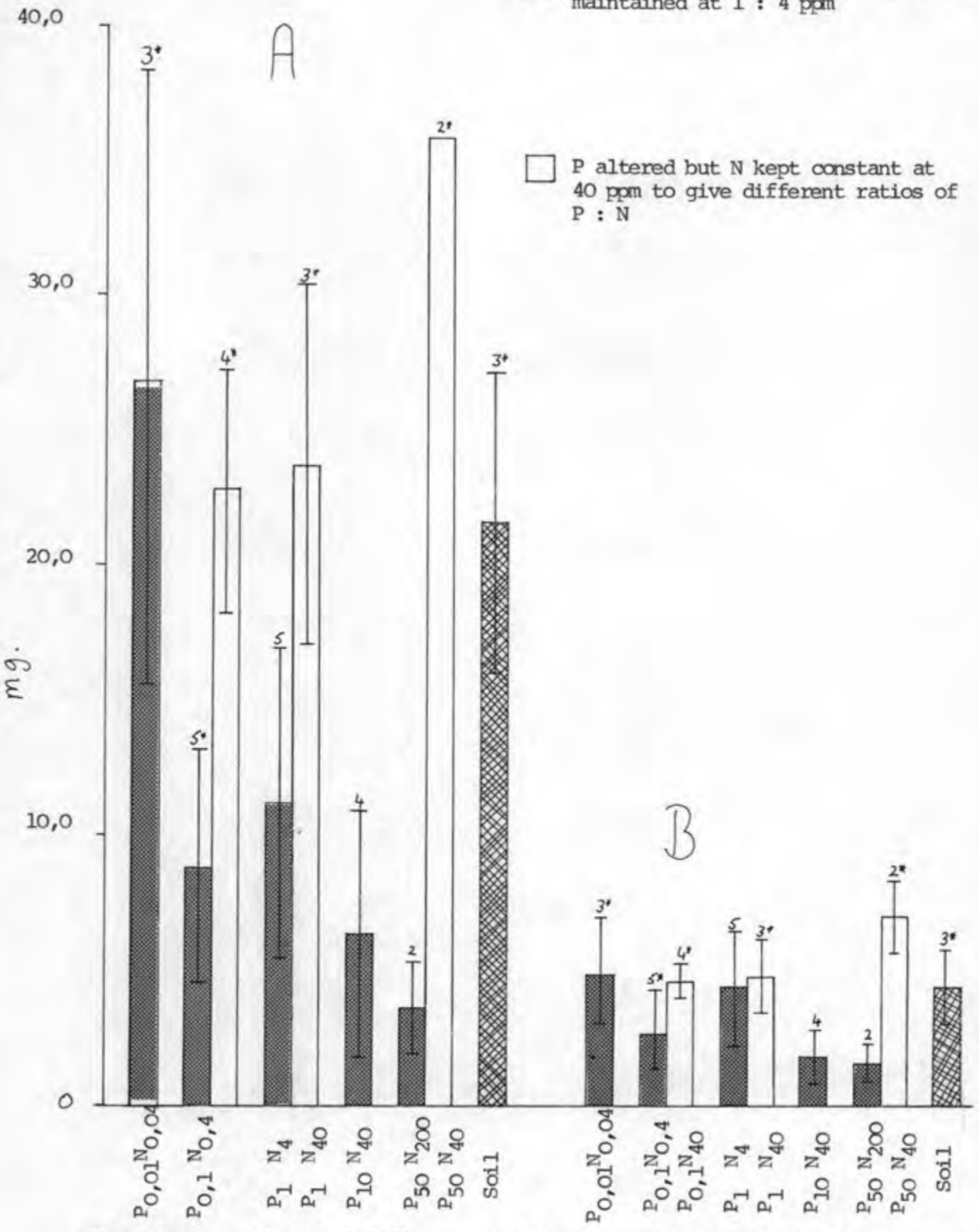

Fig. 3 : The effects of different P and N nutrient treatments on the fresh weight (A) and dry weight (B) of excised lignotubers of P. cynaroides

Note : In this and all subsequent graphs the numbers appearing above each bar represent the number of replicates per treatment. An asterisk indicates that two variants are involved.

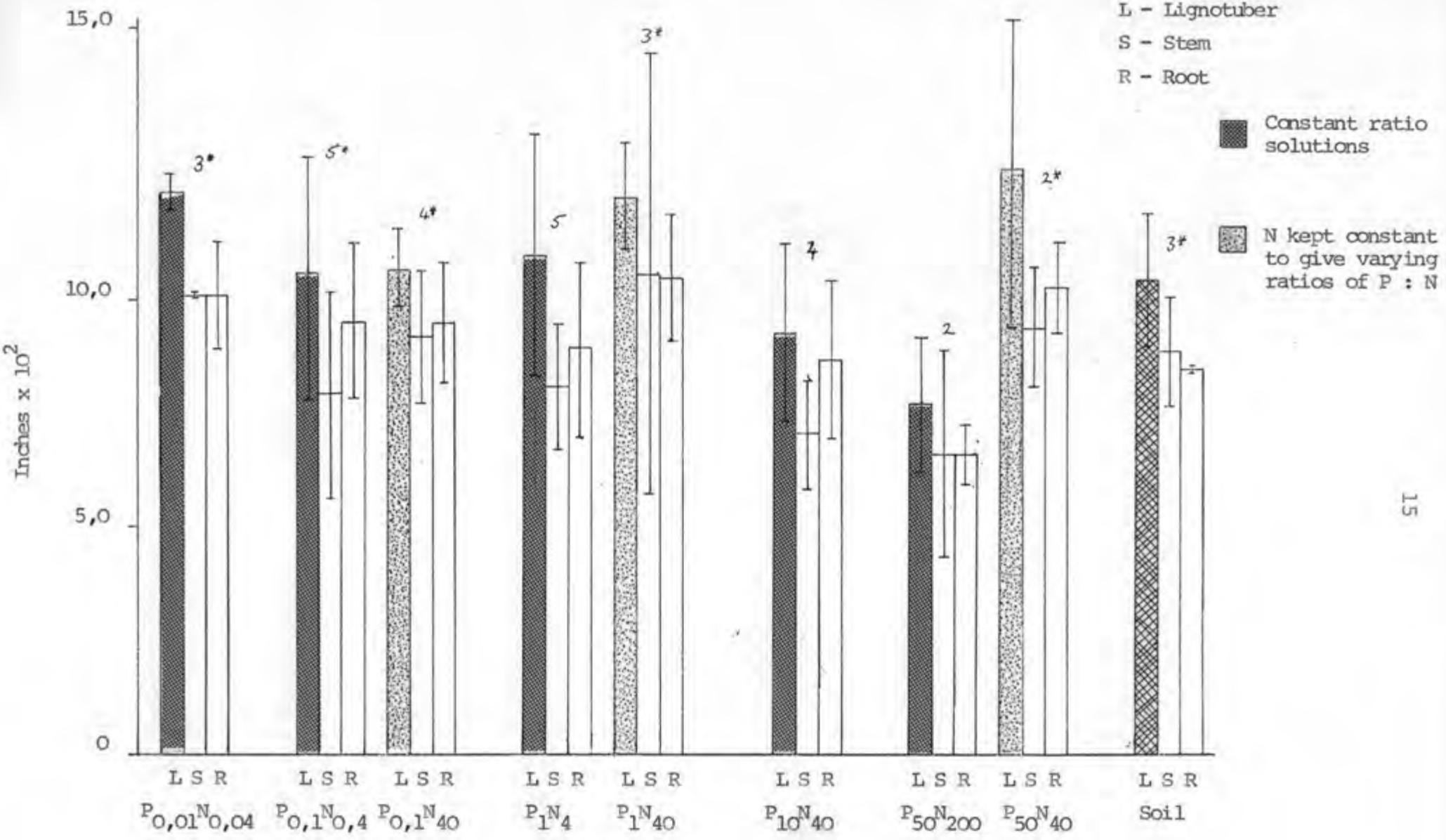

Fig. 4 : The effects of different P and N levels on th mean diameter of the lignotuber in comparison to that of stem and root 0,68 mm above and below the lignotuber respectively.

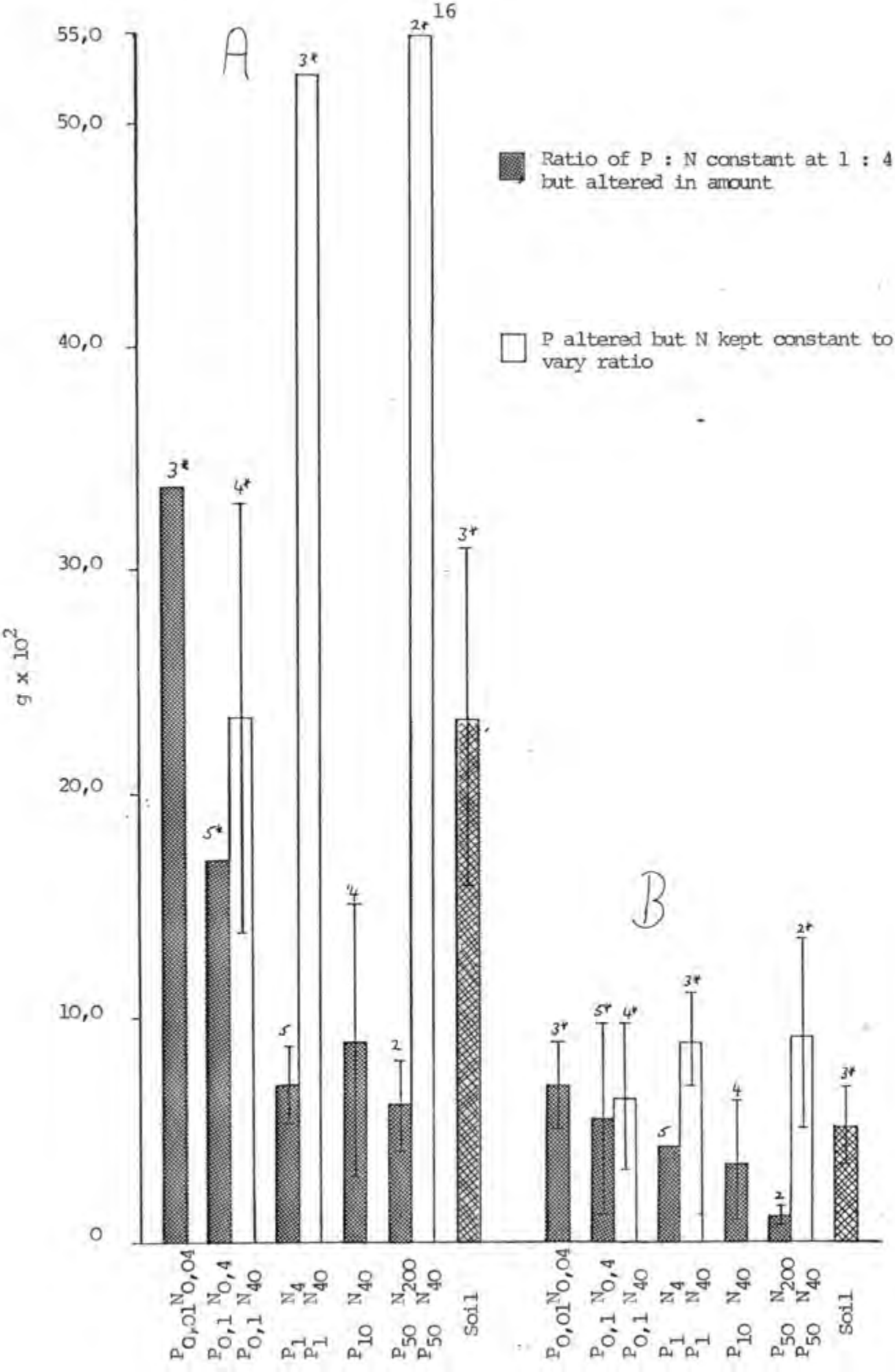

Fig. 5 : The effects of different P and N nutrient treatments on fresh weight (A) and dry weight (B) of root system below lignotuber region.

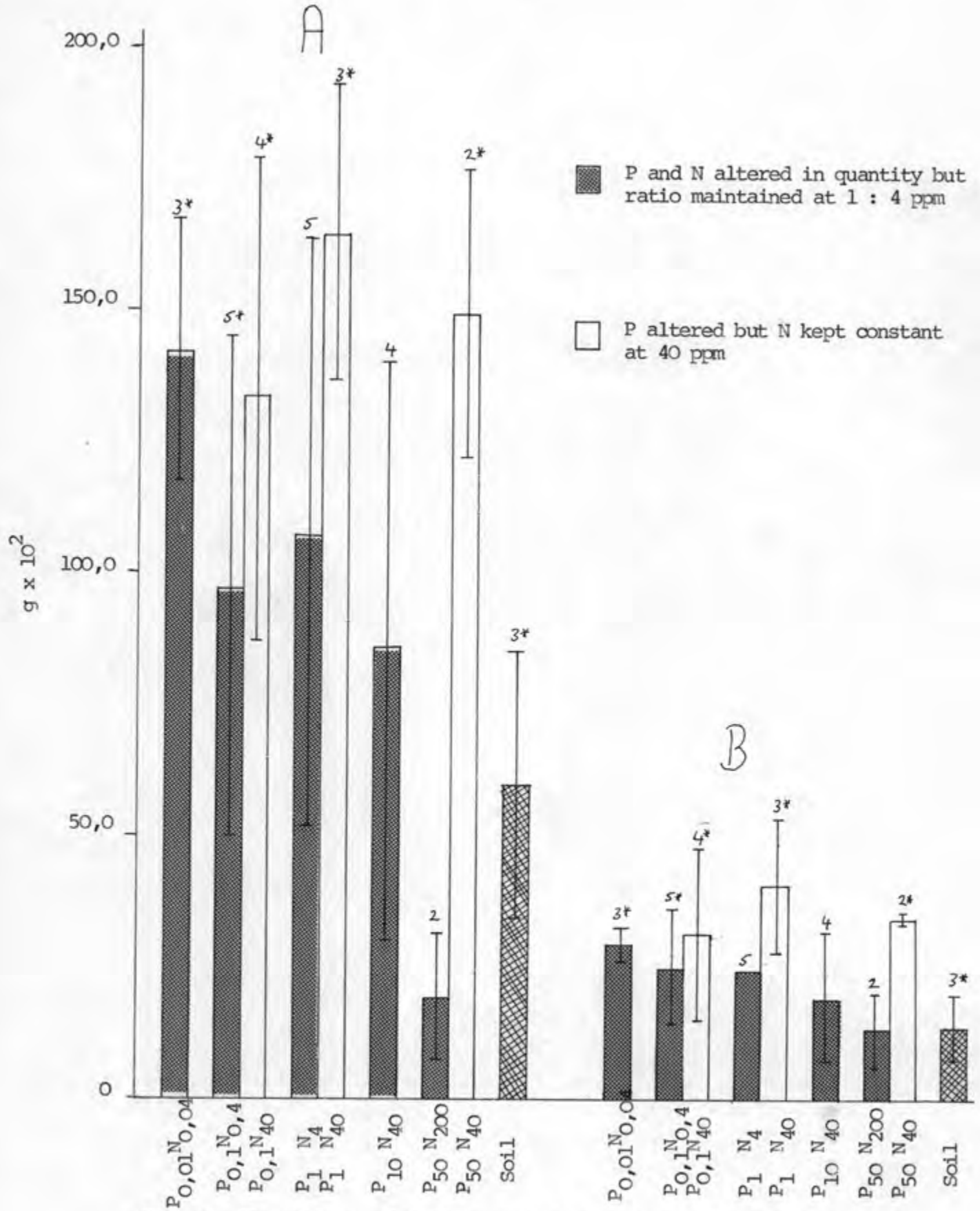

Fig. 6 : The effects of different P and N nutrient treatments on the fresh weight (A) and dry weight (B) of aerial parts above lignotuber region.

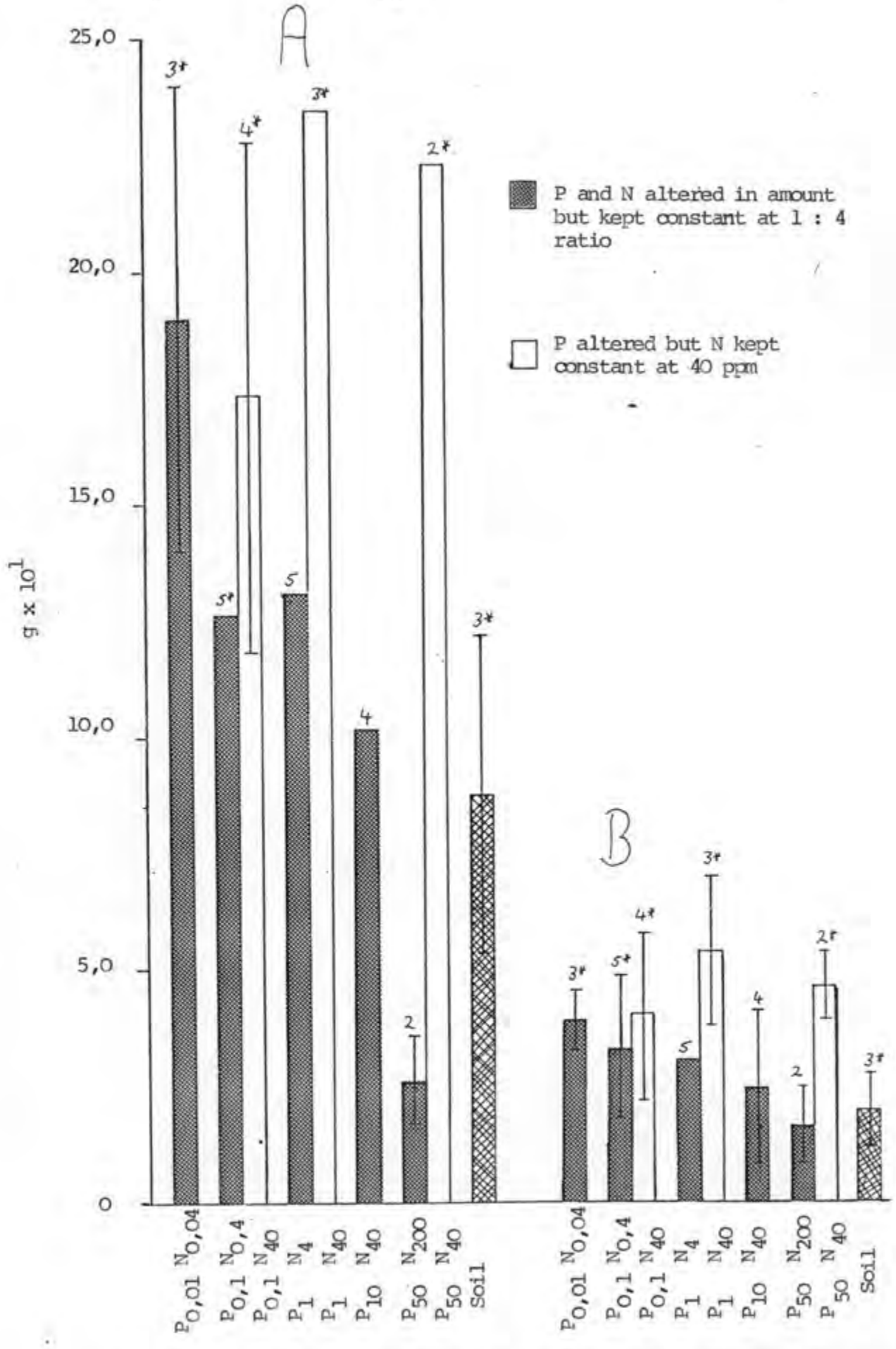

Fig. 7 : The effects of different P and N nutrient treatments on the total biomass in terms of fresh weight (A) and dry weight (B) of P. cynaroides.

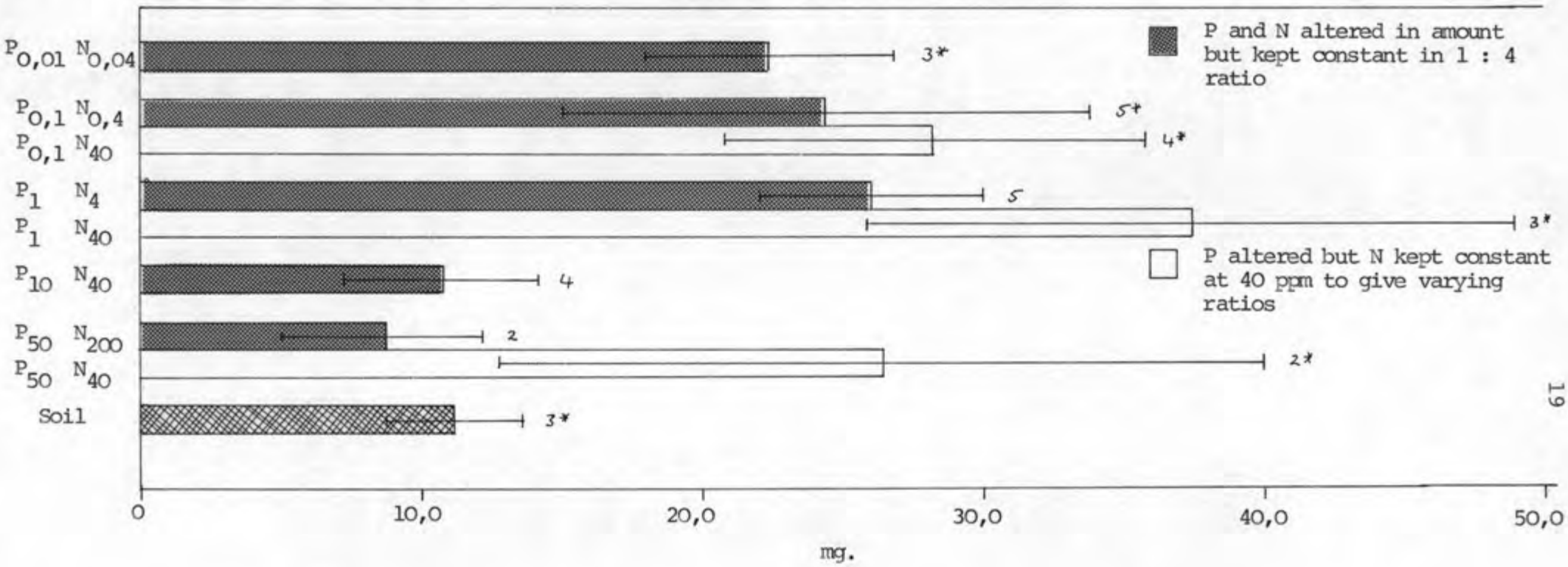

Fig. 8 : The effects of different P and N nutrient treatments on leaf area of P. cynaroides expressed in units of mass.

one case, 4 shoots with buds and small leaves. Thus, although the potential of the plants to produce lignotubers was not inhibited, their growth and activity was noticeably influenced by the concentration of P and N they received.

Leaf area as shown in Fig. 8 is shown to reflect the same trend noticed in growth rates with the greatest leaf area being found in the lower P and N treatment groups and a dramatic decline occurring in the $P_{10}N_{40}$ and $P_{50}N_{200}$ groups. A variation is noticed in that it is the P_1N_4 group which shows the greatest leaf area. This may have been due to the comparatively large sample number of 5, all of the same variant.

The varying ratio treatments, $P_{0,01}N_{40}$, P_1N_{40} and $P_{50}N_{40}$ show rather different trends to those of the constant ratio groups just discussed. Figures 5, 6 and 7 suggest that by altering the ratios of P to N we appear to have lifted the checks upon growth imposed by the 1 : 4 ratio of P to N. Whether it is actually the different ratios of P to N or the sample size which is responsible for this change in growth pattern is a matter for discussion in the following section.

The total P and NO_3^-N concentrations of the group grown in soil were calculated and are presented in Table 3. Total

TABLE 2 : THE EFFECTS OF DIFFERENT P TO N COMBINATIONS ON THE RATIO OF DRY TO FRESH WEIGHT IN *PROTEA CYNAROIDES* GROWN IN VERMICULITE MEDIUM

Treatment Group	Dry wt. expressed as % of fresh weight	Ratio of P : N	Mean %
$P_{0,01}$ $N_{0,04}$	20,5	1 : 4	23,5
$P_{0,1}$ $N_{0,4}$	25,8	1 : 4	23,5
P_1 N_4	23,5	1 : 4	23,5
P_{10} N_{40}	24,3	1 : 4	23,5
P_{50} N_{200}+	-		
$P_{0,1}$ N_{40}	23,4	1 : 400	23,4
P_1 N_{40}	23,2	1 : 40	23,2
P_{50} N_{40}	20,8	1 : 0,08	20,8
Soil	23,0		23,0

+P_{50} N_{200} not included because of wilting prior to harvesting.

TABLE 3 : TOTAL PHOSPHORUS AND NITRATES IN SOIL COLLECTED NEAR WORCESTER AND USED FOR GROWTH OF PROTEA CYNAROIDES

TOTAL PHOSPHORUS			
Sample	1	2	3
No. of Replicates	2	3	3
Mean value ug P g^{-1} soil (ppm)	254,85 ± 5,73	217,57 ±14,69	287,97 ±19,9
NITRATES			
Sample	1	2	3
No. of replicates	4	3	4
Mean value ug NO_3^- N g^{-1} soil (ppm)	13,20 ±1,5	13,00 ±0,75	15,68 ±1,5

TABLE 4 : THE EFFECTS OF DIFFERENT P AND N NUTRIENT LEVELS ON SEEDLING MORTALITY OF P. CYNAROIDES

Treatment Group	Original Sample Size	Number of Deaths	Mortality Rate %
P_{50} N_{200}	6	4	66,6
P_{50} N_{40}	6	4	66,6
P_{10} N_{40}	5	2	33,3
P_{1} N_{4}	5	0	0
P_{1} N_{40}	5	2	33,3
$P_{0,1}$ $N_{0,4}$	5	0	0
$P_{0,1}$ N_{40}	5	1	16,6
$P_{0,01}$ $N_{0,04}$	5	2	33,3
Soil	5	2	33,3

P is in the range of 250 ppm while NO_3^-N is in the range of 14 ppm. The P value does not indicate the amount of P actually available to the plant and not bound up in organic or complex form. Preliminary data (G. Brown, pers.comm.) suggests that this may be in the range of 13 ppm - 18 ppm (4% - 6% of total P). These values would theoretically correspond to a treatment of $P_{15}N_{14}$ although a direct comparison between the soil group and the controlled groups in vermiculite is not plausible. The contribution of N in other forms in the soil is not known and neither is the concentration of other major ions. Moreover, the mode of ion interaction in the soil which appeared to be a shale-sandstone mixture would not be the same as that occurring in the vermiculite medium, because of the higher percentage of colloids in the soil, for example. Suffice to say that the plants grown in soil in general grew better than the $P_{50}N_{200}$ group although not as well as the $P_{0,01}N_{0,04}$ group or any of the constant N groups. It does appear from Figures 3 and 5 that the root system of the soil group was better developed than the other groups, probably because of the greater requirement to ramify in search of nutrients.

DISCUSSION

The growth trends displayed by the constant ratio groups

tend to support the view that P. cynaroides is unable to cope with very high P and N soil concentrations, possibly because of its adaptation to fynbos soils of low nutrient status. This conclusion is substantiated by the number of mortalities which occurred in the high nutrient treatment groups comparative to the other groups (Table 4). On a much larger scale, various fertilizer studies tend to corroborate the observed trend of this study. Specht (1963) and Specht et al. (1977) have reported that although the addition of superphosphate to heathland vegetation did not affect germination, in general increased growth rates and speeded up life cycles, fertilizers did adversely affect the establishment of seedlings. Many seedlings succumbed to a 'shock effect' which seedlings of agricultural species similarly treated did not do. Moreover, many individual mature species showed signs of phosphorus toxicity although different species varied considerably in their response to increased fertility.

Notwithstanding the above supportive evidence the conclusion reached with regard to the present study cannot be regarded as definitive without first discussing a number of ideas.

The enhanced growth in the varying ratio groups (N kept constant at 40 ppm) tends to suggest that high levels of P and N in the ratio of 1 : 4 are detrimental to growth

whereas by altering the ratio, the high levels become less detrimental. There is evidence to suggest that sclerophyllous species may vary considerably in their response to increasing levels of P and N. Groves and Keraitis (1975), for example, report that Banksia serrata seedlings were intolerant of a combination of high P and N levels, whereas seedlings of Acacia suaveolens died at high P levels irrespective of the N level and Eucalyptus pilularis seedlings were intolerant of high P or high N alone, but responded to high P levels combined with high N levels. The authors concluded that over a wide range of P levels, growth of sclerophyll species was increased by additions of P up to at least 1 ppm, but that growth responses above this level depended largely on differences between the species, especially with respect to seed size and the level of soil N associated with the increased P. In the context of the present study it is possible that just such a situation arose; above a certain level of P and N (0,1 ppm P, 0,4 ppm N) the ratio of 1 : 4 was detrimental to growth, this effect being inherent in the genetic constitution of the species concerned. By altering the ratio of P : N the inhibiting effect on growth was relieved to some extent, but only up to a maximum ($P_{50}N_{40}$) when deaths from toxicity resulted irrespective of the P : N ratio.

It is interesting to consider a phenomenon known as Macy's theory (1936, in Thompson, 1952). Macy observed that when an element is deficient and a small addition is made there is a dramatic increase in growth without an increase in percentage composition of the element in the tissues. With increasing additions of the deficient element the increase in growth levels off rapidly and the percentage composition of the element increases. With regard to our present study this effect could have been present over which may have been superimposed an inhibiting effect at high levels caused by a specific P : N ratio.

In considering confounding variables it is possible that the two variants of P. cynaroides might vary in their relative tolerances to high P and N nutrient treatments and in their preferences for a particular ratio. Although this variable might not affect the trends obtained as such, they would affect absolute values which are amplified by the small sample size (Appendix 1). Seed size differences and therefore differences in mineral reserve amounts between the two variants and between seeds of the same variant, although not noticed or even looked for at the time of sowing, cannot be discarded as confounding variables, although at 14 weeks old these differences had probable disappeared. One must also consider that the concentrations of Ca and Na ions differed in the various treatment groups

(Table 1). Although the highest and lowest Ca levels used are within the range used in hydroponic solutions (Harris, 1966), Na was very low in some groups and so the balance of salts other than those under control differed in the different groups so increasing the range of possible ionic interactions and the number of uncontrolled variables.

Although deficiency symptoms such as chlorosis and the development of necrotic leaf tips and margins were observed, they were not peculiar to any specific treatment group. They most likely resulted from a general inefficiency in mobilizing nutrients which appears to be common to many Proteaceae in the seedling stage (Shütte, pers.comm.). Thus despite the definite trend in growth response which emerged from this study, and despite the fact that explanations to account for this trend are forthcoming, the number of unknown and therefore uncontrolled variables does not allow one to generalize to any definitive degree.

Support for a nutritional interpretation of sclerophylly as advanced by Loveless (1961, 1962) tends to be given by fresh to dry weight ratios. Table 2 indicates that a high P to N ratio the percentage of the total mass which is non-living (i.e. fibrous) is less than at all lower P levels irrespective of the ratio. At lower P levels therefore

less protein may be being formed with intermediate metabolites being diverted to form fibrous material, as proposed by Loveless (1961).

As indicated in the previous section, the potential of the plant to develop a lignotuber does not appear to be affected by soil nutrient status. The growth of the lignotuber tends to follow that of the rest of the plant and in this sense is affected by the amount of available nutrients. The activity of the lignotuber in terms of shoot growth, however, does not reflect the general growth trend of the plant but reflects a direct response to the level of nutrients opposite to that of the plant as a whole. Thus activity may be a more useful parameter to measure lignotuber dynamics.

This discussion would not be complete without suggesting some of the improvements which could be implemented in a future controlled study of this type. Firstly, it seems desirable to approximate more closely the natural edaphic environment of sclerophyllous fynbos species. Possible differences in variables like drainage capacity, ion adsorption capacity, soil texture and particle size would then be less pronounced. It is suggested therefore that acid-washed sand should be used to approximate the texture and physical qualities of Table Mountain sandstone.

Secondly, the concentration of salts in the nutrient solutions (other than those under manipulation) should perhaps be in the same range of concentrations as found in the normal edaphic environment of the plant. To date some idea of the concentration of major ions in fynbos soils is known (B. Low, unpublished data) and these values can be used to modify standard hydroponic solutions or stock solutions especially low in nutrients, e.g. Robbin's solution (Robbins and White, 1936). The addition of supplementary salts may be necessary in order to maintain levels of secondary ions altered by manipulation of major ions.

Thirdly, in order to reduce the complex interactions which arise when too many variables are being manipulated, it might be wiser to manipulate only one nutrient at a time, e.g. P alone in one group of plants, N alone in another and only then P and N together. In this way the individual as well as the composite effects of the major nutrients can be defined. It also follows from this project that seeds which are easier to germinate under artificial conditions should be used (e.g. Protea repens, Leucadendron sélignum) and that these seeds should all come from the same population of parent plants. Initial sorting of the seeds with regard to size and maturity would also be advisable.

This project, although encountering a number of unexpected

problems, has succeeded in demonstrating the potential of this type of study in elucidating the growth trends of indigenous shrubs. With greater refinements and corroboration from field studies, studies of this type must ultimately help to clarify the adaptive dynamics of the unique Cape floral kingdom.

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APPENDIX 1. Table to compare Variant 1 (broad-leaved), Variant 2 (narrow-leaved) and mean total biomass of *Protea cynaroides*.

FRESH WEIGHT.			
Sample	Variant 1 ($g \times 10^2$)	Variant 2 ($g \times 10^2$)	Mean of both variants.
$P_{0,01}$ $N_{0,04}$	163,8	247,6	190,2
$P_{0,1}$ $N_{0,4}$	164,9	68,9	126,5
$P_{0,1}$ N_{40}	155,1	228,1	173,4
P_1 N_{40}	263,9	176,0	234,5
P_{50} N_{40}	168,7	276,1	222,4
DRY WEIGHT			
Sample	Variant 1 ($g \times 10$	Variant 2 ($g \times 10$	Mean of both variants
$P_{0,01}$ $N_{0,04}$	35,4	109,6	39,1
$P_{0,1}$ $N_{0,4}$	42,4	17,9	32,6
$P_{0,1}$ N_{40}	33,16	62,9	40,6
P_1 N_{40}	63,6	35,8	54,3
P_{50} N_{40}	41,6	51,08	46,4

APPENDIX 2 : RAW DATA.

TREATMENT: $P_{0,01}$ $N_{0,04}$

LIGNOTUBERS.

Sample	Fresh wt. (mg)	Dry Wt. (mg)	Diameters (ins. X^{-1} 10^2).
1	16,4	3,5	12,4
2	39,0	7,3	12,8
3	25,3	4,0	12,0
4	—	—	—
5	—	—	—
	$\bar{X}$ = 26,9 S.D = ±11,38	$\bar{X}$ = 4,9 S.D = ±2,06	$\bar{X}$ = 12,4 S.D = ±0,40

ROOTS

Sample	Fresh wt. (g X10^2)	Dry Wt. (g X10^2)	Diameters (ins. X10^2)
1	61,9	10,7	11,5
2	20,3	5,9	9,3
3	19,4	4,7	9,5
4	—	—	—
5.	—	—	—
	$\bar{X}$ = 33,76 S.D = ±24,09	$\bar{X}$ = 6,39 S.D = ±3,19	$\bar{X}$ = 10,1 S.D = ±1,22.

AERIAL PARTS

Sample	Fresh wt. (g X10^2)	Dry wt. (g X10^2)	Diameters (ins. X10^2)
1	184,4	35,3	10,0
2	145,2	29,5	10,1
3	128,02	29,6	10,0
4	—	—	—
5	—	—	—
	$\bar{X}$ = 162,6 S.D = ±25,01	$\bar{X}$ = 32,4 S.D = ±3,03	$\bar{X}$ = 10,03 S.D = ± 0,06

TOTAL MEAN BIOMASS.

	Fresh wt. (g X10^2)	Dry wt. (g X10^2)	
	$\bar{X}$ = 190,2 S.D = ± 50,4	$\bar{X}$ = 39,1 S.D = ±6,4	

APPENDIX 3 : RAW DATA.

TREATMENT: $P_{0,1}$ $N_{0,4}$

LIGNOTUBERS.			
Sample	Fresh wt. (mg)	Dry Wt. (mg)	Diameters (ins. $\times 10^2$).
1	12,1	4,9	13,9
2	6,8	2,4	11,6
3	14,0	3,2	12,0
4	4,1	1,3	7,6
5	5,8	1,5	7,7
	$\bar{X}$ = 8,6 S.D = ± 4,3	$\bar{X}$ = 2,7 S.D = ± 1,4	$\bar{X}$ = 10,6 S.D = ±1,2

ROOTS			
Sample	Fresh wt. (g $\times 10^2$)	Dry Wt. (g $\times 10^2$)	Diameters (ins. $\times 10^2$)
1	44,7	12,4	12,3
2	13,3	5,3	10,0
3	13,5	5,4	8,7
4	8,4	2,8	8,7
5.	5,3	1,8	7,8
	$\bar{X}$ = 17,02 S.D = ± 5,4	$\bar{X}$ = 5,5 S.D = ± 4,0	$\bar{X}$ = 9,5 S.D = ± 1,8

AERIAL PARTS			
Sample	Fresh wt. (g $\times 10^2$)	Dry wt. (g $\times 10^2$)	Diameters (ins. $\times 10^2$)
1	191,1	41,2	9,9
2	126,1	28,7	9,2
3	102,7	33,3	9,5
4	71,9	18,61	5,2
5	51,1	12,4	5,6
	$\bar{X}$ = 100,8 S.D = ±48,3.	$\bar{X}$ = 24,94 S.D = ± 10,2.	$\bar{X}$ = 7,88 S.D = ± 2,9

TOTAL MEAN BIOMASS.		
	Fresh wt. (g $\times 10^1$)	Dry wt. (g $\times 10^1$)
	$\bar{X}$ = 126,48 S.D = ± 66,5.	$\bar{X}$ = 32,62 S.D = ± 15,5

APPENDIX 4 : RAW DATA.

TREATMENT: $P_{0,1}$ N_{40}

LIGNOTUBERS.

Sample	Fresh wt. (mg)	Dry Wt. (mg)	Diameters (ins. X 10^2).
1	22,6	4,4	11,0
2	21,5	4,1	10,8
3	27,7	5,5	11,5
4	16,8	4,2	9,5
5	—	—	—
	$\bar{X}$ = 22,20 S.D = ± 4,4	$\bar{X}$ = 4,55 S.D = ± 0,7	$\bar{X}$ = 10,70 S.D = ± 0,9

ROOTS

Sample	Fresh wt. (g X 10^2)	Dry Wt. (g X 10^2)	Diameters (ins. X 10^2)
1	23,2	6,5	8,9
2	33,7	7,9	10,6
3	26,2	7,7	10,5
4	10,5	3,5	7,8
5.	—	—	—
	$\bar{X}$ = 23,42 S.D = ± 9,7	$\bar{X}$ = 7,12 S.D = ± 2,0	$\bar{X}$ = 9,45 S.D = ± 1,4

AERIAL PARTS

Sample	Fresh wt. (g X 10^2)	Dry wt. (g X 10^2)	Diameters (ins. X 10^2)
1	141,3	25,2	9,9
2	163,1	37,8	11,0
3	199,1	54,8	8,0
4	87,4	17,4	8,0
5	—	—	—
	$\bar{X}$ = 164,85 S.D = ± 44,6	$\bar{X}$ = 33,8 S.D = ± 16,0	$\bar{X}$ = 9,23 S.D = ± 1,5

TOTAL MEAN BIOMASS.

	Fresh wt. (g X 10^1)	Dry wt. (g X 10^1)	
	$\bar{X}$ = 173,36 S.D = ± 55,1	$\bar{X}$ = 40,62 S.D = ± 18,0	

APPENDIX 5 : RAW DATA.

TREATMENT: $P_1 N_4$

LIGNOTUBERS.

Sample	Fresh wt. (mg)	Dry Wt. (mg)	Diameters (ins. x 10^2).
1	7,9	3,5	9,0
2	14,1	4,3	11,2
3	17,9	3,8	13,4
4	5,2	2,2	7,6
5	28,8	8,0	13,8
	$\bar{X}$ = 14,80 S.D = ±5,8	$\bar{X}$ = 4,36 S.D = ±2,1	$\bar{X}$ = 11,00 S.D = ±2,7

ROOTS

Sample	Fresh wt. (g x 10^2)	Dry Wt. (g x 10^2)	Diameters (ins. x 10^2)
1	9,1	5.5	8,8
2	5,3	3,2	10,1
3	7,6	3,7	8,5
4	6,1	2,6	6,0
5.	27,7	10,7	11,2
	$\bar{X}$ = 11,1 S.D = ±1,7	$\bar{X}$ = 4,2 S.D = ±1,4	$\bar{X}$ = 8,9 S.D = ±1,9

AERIAL PARTS

Sample	Fresh wt. (g x 10^2)	Dry wt. (g x 10^2)	Diameters (ins. x 10^2)
1	123,7	29,7	7,6
2	99,2	22,1	8,0
3	71,1	17,7	9,6
4	76,0	15,5	6,1
5	221,3	46,1	9,2
	$\bar{X}$ = 118,7 S.D = ±23,1	$\bar{X}$ = 26,2 S.D = ±5,9	$\bar{X}$ = 8,1 S.D = 1,3

TOTAL MEAN BIOMASS.

	Fresh wt. (g x 10^1)	Dry wt. (g x 10^1)	
	$\bar{X}$ = 130,8 S.D = ±70,8	$\bar{X}$ = 30,8 S.D = ±15,7.	

APPENDIX 6 : RAW DATA.

TREATMENT: $P_1 N_{40}$

LIGNOTUBERS.

Sample	Fresh wt. (mg)	Dry Wt. (mg)	Diameters (ins. x 10^2).
1	31,3	6,2	13,1
2	21,6	3,9	12,9
3	18,2	3,9	10,9
4	–	–	–
5	–	–	–
	$\bar{X}$ = 23,7 S.D = ± 6,8	$\bar{X}$ = 4,8 S.D = ± 1,3	$\bar{X}$ = 12,3 S.D = ± 1,2

ROOTS

Sample	Fresh wt. (g x 10^2)	Dry Wt. (g x 10^2)	Diameters (ins. x 10^2)
1	72,4	10,2	9,2
2	54,3	10,2	10,3
3	29,9	6,7	12,0
4	–	–	–
5.	–	–	–
	$\bar{X}$ = 52,2 S.D = ± 21,3	$\bar{X}$ = 9,0 S.D = ± 2,0	$\bar{X}$ = 10,5 S.D = ± 1,4

AERIAL PARTS

Sample	Fresh wt. (g x 10^2)	Dry wt. (g x 10^2)	Diameters (ins. x 10^2)
1	195,9	53,8	13,0
2	199,7	52,1	12,8
3	144,3	28,8	8,4
4	–	–	–
5	–	–	–
	$\bar{X}$ = 171,05 S.D = ± 27,8	$\bar{X}$ = 40,9 S.D = ± 12,2	$\bar{X}$ = 10,6 S.D = ± 4,9

TOTAL MEAN BIOMASS.

	Fresh wt. (g x 10^1)	Dry wt. (g x 10^1)	
	$\bar{X}$ = 234,5 S.D = ± 51,3	$\bar{X}$ = 54,3 S.D = ± 16,1	

APPENDIX 7 : RAW DATA.

TREATMENT: P_{10} N_{40}

LIGNOTUBERS.

Sample	Fresh wt. (mg)	Dry Wt. (mg)	Diameters (ins. X 10^2).
1	2,3	1,0	7,9
2	2,8	1,1	7,6
3	8,5	2,3	9,5
4	11,9	3,1	12,0
5	—	—	—
	$\bar{X}$ = 6,4 S.D = ± 4,6	$\bar{X}$ = 1,9 S.D = ± 1,0	$\bar{X}$ = 9,3 S.D = ± 2,0

ROOTS

Sample	Fresh wt. (g X10^2)	Dry Wt. (g X10^2)	Diameters (ins. X10^2)
1	4,4	1,4	8,0
2	4,5	1,7	7,1
3	9,1	3,2	9,1
4	17,6	7,4	10,5
5.	—	—	—
	$\bar{X}$ = 8,88 S.D = ± 2,7	$\bar{X}$ = 3,44 S.D = ± 1,8	$\bar{X}$ = 8,7 S.D = ± 1,5

AERIAL PARTS

Sample	Fresh wt. (g X10^2)	Dry wt. (g X10^2)	Diameters (ins. X10^2)
1	44,0	15,8	5,8
2	70,4	28,6	6,8
3	73,9	14,4	7,1
4	180,2	40,8	8,7
5	—	—	—
	$\bar{X}$ = 89,6 S.D = ± 23,1	$\bar{X}$ = 21,1 S.D = ± 5,0	$\bar{X}$ = 7,1 S.D = ± 1,2

TOTAL MEAN BIOMASS.

	Fresh wt. (g X10^1)	Dry wt. (g X10^1)	
	$\bar{X}$ = 101,6 S.D = ± 74,06	$\bar{X}$ = 24,7 S.D = ± 16,5.	

APPENDIX 8 : RAW DATA.

TREATMENT: P_{50} N_{200}

LIGNOTUBERS.

Sample	Fresh wt. (mg)	Dry Wt. (mg)	Diameters (ins. X 10^2).
1	4,8	2,0	8,7
2	2,4	1,1	6,6
3	-	-	-
4	-	-	-
5	-	-	
	$\bar{X}$ = 3,6 S.D = ±1,7	$\bar{X}$ = 1,55 S.D = ±0,64.	$\bar{X}$ = 7,70 S.D = ±1,5

ROOTS

Sample	Fresh wt. (g X 10^2)	Dry Wt. (g X 10^2)	Diameters (ins. X 10^2)
1	4,66	2,02	7,0
2	7,51	1,97	6,1
3	-	-	-
4	-	-	-
5.	-	-	-
	$\bar{X}$ = S.D =	$\bar{X}$ = S.D =	$\bar{X}$ = S.D =

AERIAL PARTS

Sample	Fresh wt. (g X 10^2)	Dry wt. (g X 10^2)	Diameters (ins. X 10^2)
1	27,85.	19,19	8,2
2	11,70	9,31.	5,0
3	-	-	-
4	-	-	-
5	-	-	-
	$\bar{X}$ = 19,78 S.D = ±11,9	$\bar{X}$ = 14,26 S.D = ± 7,4	$\bar{X}$ = 6,6 S.D = ±2,3.

TOTAL MEAN BIOMASS.

	Fresh wt. (g X 10^1)	Dry wt. (g X 10^1)	
	$\bar{X}$ = 26,22 S.D = ± 9,5	$\bar{X}$ = 16,40 S.D = ± 7,1	

APPENDIX 9 : RAW DATA.

TREATMENT: P_{50} N_{40}

LIGNOTUBERS.

Sample	Fresh wt. (mg)	Dry Wt. (mg)	Diameters (ins. X 10^2).
1	12,9	2,9	10,5
2	58,9	11,0	15,4
3	–	–	–
4	–	–	–
5	–	–	–
	$\bar{X}$ = 35,9 S.D = —	$\bar{X}$ = 6,9 S.D = ± 1,3	$\bar{X}$ = 12,9 S.D = ± 3,4

ROOTS

Sample	Fresh wt. (g X 10^2)	Dry Wt. (g X 10^2)	Diameters (ins. X 10^2)
1	28,4	5,9	9,6
2	85,2	12,3	11,0
3	–	–	–
4	–	–	–
5.	–	–	–
	$\bar{X}$ = 56,8 S.D = —	$\bar{X}$ = 9,2 S.D = —	$\bar{X}$ = 10,3 S.D = ± 0,9

AERIAL PARTS

Sample	Fresh wt. (g X 10^2)	Dry wt. (g X 10^2)	Diameters (ins. X 10^2)
1	139,0	35,3	8,4
2	185,0	37,7	10,3
3	–	–	–
4	–	–	–
5	–	–	–
	$\bar{X}$ = 162,0 S.D = ± 27,5	$\bar{X}$ = 36,5 S.D = ± 1,0	$\bar{X}$ = 9,4 S.D = ± 1,3

TOTAL MEAN BIOMASS.

	Fresh wt. (g X 10^1)	Dry wt. (g X 10^1)	
	$\bar{X}$ = 222,4 S.D = ± 76,0	$\bar{X}$ = 46,6 S.D = ± 6,7	

APPENDIX 10 : RAW DATA.

TREATMENT: SOIL.

LIGNOTUBERS.

Sample	Fresh wt. (mg)	Dry Wt. (mg)	Diameters (ins. X 10^2).
1	14,8	2,8	8,6
2	26,0	5,4	11,4
3	23,9	5,1	11,0
4	–	–	–
5	–	–	–
	$\bar{X}$ = 21,6 S.D = ± 5,9	$\bar{X}$ = 4,4 S.D = ± 1,4	$\bar{X}$ = 10,5 S.D = ± 1,5

ROOTS

Sample	Fresh wt. (g X 10^2)	Dry Wt. (g X 10^2)	Diameters (ins. X 10^2)
1	12,7	3,5	8,5
2	27,6	6,9	8,5
3	23,1	5,1	9,6
4	–	–	–
5.	–	–	–
	$\bar{X}$ = S.D =	$\bar{X}$ = S.D =	$\bar{X}$ = S.D =

AERIAL PARTS

Sample	Fresh wt. (g X 10^2)	Dry wt. (g X 10^2)	Diameters (ins. X 10^2)
1	33,7	7,2	7,5
2	70,0	16,1	9,5
3	88,7	20,2	8,4
4	–	–	–
5	–	–	–
	$\bar{X}$ = 70,3 S.D = ± 26,9	$\bar{X}$ = 15,9 S.D = ± 6,4	$\bar{X}$ = 8,9 S.D = ± 1,2

TOTAL MEAN BIOMASS.

	Fresh wt. (g X 10^1)	Dry wt. (g X 10^1)	
	$\bar{X}$ = 87,4 S.D = ± 34,9	$\bar{X}$ = 20,1 S.D = ± 8,0	