

Thesis
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**Ecological Investigations on Selected Species at the Meikle Kilrannoch
Ultramafic Outcrops, Scotland**

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by

Laszlo Nagy

Department of Biological and Molecular Sciences,

University of Stirling.

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"I am quite convinced that long long ago my ancestors were dwellers among the wilds of the mountains, for at times I have an almost insatiable yearning for the hills. Indeed, they always enchanted me; and, when a small child in a remote part of the Highlands, I loved them so dearly that I carefully selected the mountains which appealed to me most. And since that time I have reserved them strictly for my own private thoughts and fitful dreams. Of course I visit them at odd intervals in order to make certain that everything there is as it ought to be."

(from My Private Alps, in Behold the Hebrides by Alasdair Alpin McGregor)

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Abstract

Ecological and ecophysiological investigations carried out at the ultramafic outcrop near Meikle Kilrannoch, Angus, Scotland are reported. The outcrop is botanically famous for its rare plant species, particularly the endemic Cerastium fontanum ssp. scoticum and the nationally rare Lychnis alpina. The studies were made on the main outcrop (called MK1) which is dome shaped, and on a much smaller low-lying area (called MK1.5) about 300 m from it.

The overall aim of the studies was to investigate the relationship between the soil physico-chemical environment and species distribution on the open areas of the ultramafic site and to experimentally test for causality; and to offer an explanation for the open character of the vegetation on the skeletal soils.

Variograms which were constructed for soil properties and vegetation data to investigate soil micro-spatial variation and vegetation pattern showed differing levels of spatial dependence, always indicating high intrinsic variability. The cause of this high variability was probably cryoturbation for the soil and morphological characters for plants.

The gradient analyses (Principal Components Analysis and its canonical form, Redundancy Analysis) used to study soil - vegetation correlations suggested that Agrostis vinealis, Cerastium fontanum ssp. scoticum and Lychnis alpina were most abundant in areas up-slope with lower concentrations of soil magnesium; Cochlearia pyrenaica ssp. alpina and Festuca rubra were associated with bigger stone sizes, and the latter occurred in wetter areas with higher availability of ions.

A comparative solution culture experiment based on the local soil chemistry was used to study the growth responses to magnesium and nickel of Cerastium fontanum ssp.

scoticum, Cochlearia pyrenaica ssp. alpina and Festuca rubra. The results for Festuca and Cerastium were in agreement with the findings of the gradient analysis: Festuca was indifferent to both magnesium and nickel and Cerastium was susceptible to high magnesium; the reduction of dry weight by nickel in the Cochlearia conflicted with its suggested association with high soil nickel in the gradient analysis.

The impacts on the photosynthetic systems of three Cochlearia species of different concentrations of iron and nickel were identifiable only in the non-ultramafic C. pyrenaica where the addition of nickel decreased photosynthesis but the effect could be ameliorated by the addition of high concentrations of iron.

The open character of the skeletal soil at the MK1 site was discussed in terms of 'carrying capacity'. Vegetation development was suggested to be controlled at least partly by large stones covering the soil surface. Further factors such as space fragmentation, possible plant-to-plant interactions, and low density of flowering individuals and restricted seed dispersal were also considered.

To test if major nutrients were limiting plant growth, major nutrients (NPK) were applied to the MK1.5 skeletal soil. The significantly higher λ 's and recruitment and change in life history traits (larger rosette sizes, earlier maturing and higher seed production) in the fertilised populations of C. pyrenaica ssp. alpina resulted in a significantly higher plant cover in the fertilised quadrats. The better growth of plants in the fertilised quadrats was reflected in their lower total non-structural carbohydrate (TNC) concentrations in May and their higher TNC in August.

The present series of investigations found that magnesium and nickel toxicity had an effect on the intra-site distribution of the ultramafic species and also confirmed earlier reports on the importance of magnesium and nickel toxicity in ultramafic exclusion. Large stones and low soil phosphorus concentration are proposed as limiting factors for

the development of closed vegetation on the skeletal soil areas of the sites.

Chapter I

Introduction and background to the study site

The term 'ultramafic' refers to ferromagnesian, dark-coloured igneous or metamorphic rocks and is being increasingly used in the botanical and ecological literature to replace the formerly popular terms 'serpentine' and 'ultrabasic'. This has occurred because of the discrepancy between the meaning of 'serpentine' in its correct sense to refer to certain types of minerals and its popular botanical usage for soils and vegetation developed over ultramafic rocks which may or may not contain the true serpentine minerals; and the fact that 'ultrabasic' emphasises the relatively low silicon concentration in the rocks and includes some rocks which are not ultramafic. In this thesis ultramafic is used in preference to 'serpentine' and 'ultrabasic' throughout.

Many ultramafic rocks have been serpentinised, the name given to the metamorphic process whereby ultramafic protoliths become hydrated under relatively low pressure and temperature (<500 °C). The cause of the process is the interaction of the rock with water (sea, meteoric and hydrothermal). The protoliths are mostly ophiolite fragments of the oceanic crust and mantle exposed as a result of intense tectonic activity. Hence serpentinised ultramafics are common in fold mountain belts (Coleman & Jove 1992, Malpas 1992). After serpentinisation the general composition of minerals which make up the ultramafic rocks is $Mg_3Si_2O_5(OH)_4$; Fe and Ni may substitute some, or all of the Mg and some of the Si. The chemical composition of the ultramafic rocks is often reflected in the composition of the soils overlying them (Table 1.1).

%	P	K	Ca	Mg	Fe	Ni	Si
u.m.	0.01	0.01	0.01	15.6	13.7	0.47	34
n-u.m.	0.17	1.8	1.61	0.92	3.8	-	70

Table 1.1 An example of the total concentrations of elements of soils derived from ultramafic (u.m.) and non-ultramafic (n-u.m.) parent rock (Brooks 1987).

Ultramafics often have a distinctive vegetation which contrasts with that of the surrounding areas, depending on the precise composition of the rock, the chemical and physical attributes of the soils derived from it and the neighbouring geology. Frequently the vegetation on temperate ultramafics has the following features: low cover, low stature, and the presence of rare or endemic taxa. The causes of the features have received various explanations: excess magnesium (absolute and relative to calcium); generally low major nutrient status; high nickel; and physical features such as shallow, coarse-textured soils, which are prone to drought.

The long history of botanical and ecological research on ultramafics has been reviewed extensively (e.g. Brooks 1987, Roberts & Proctor 1992). A compendium of recent papers on ultramafics was published by Baker *et al.* (1992) and includes an overview on the current state of research on ultramafics which has been co-authored by the writer of this thesis (Proctor & Nagy 1992) and which is appended (Appendix II).

Ultramafics occur in many places in Scotland (Geological Survey of Scotland 1962). Those researched most botanically: in Ayrshire, Central Scotland, Aberdeenshire, Rhum and the Shetland Islands have been described by Proctor (1992).

The field studies presented here were carried out at the ultramafic outcrop near the hill called Meikle Kilrannoch, Angus (Fig. 1.1). Two sites near Meikle Kilrannoch: 'MK1' (National Grid Ref. 219777) and MK2 (NGR 220768) of the five ultramafic sites at the head of Glen Doll (Geographical Survey of Scotland 1962) have been ecologically researched since Marshall (1959) and much of the work there and on the recently exposed ultramafic area 'MK1.5' is summarised in Proctor *et al.* (1991).

The sites lie at about 870 m elevation above sea level. MK1 is about 3.5 ha in extent and is dome-shaped rising above the surrounding blanket bog while MK2 is a 2.5 ha, largely flat area. No studies were made at MK2 during the present work. The study site, MK 1.5 is a small area of about 400-500 m² of exposed skeletal soil surrounded by vegetated and eroding peat and it is about 300 m south-west of the southern point of MK1. MK 1.5 was exposed by peat erosion between 1946 and 1966 according to aerial photographs of the area. The site is flat and low lying and therefore prone to high soil water table levels and water may collect after heavy rains, or snow melt.

Oceanic influence in the area of Meikle Kilrannoch is less pronounced than in most other parts of highland Scotland (Brown *et al.* 1993) and the occurrence of alpine species nearby is an indication of the influence of a less-harsh version of a continental alpine type climate. For average weather data Proctor *et al.* (1991) presented estimated values based on extrapolations from the data collected from Braemar meteorological station (20 km to the north of the sites at an altitude of 340 m) between 1941-1970. The mean annual precipitation was estimated to be c. 1300 mm (879 mm at Braemar); mean daily maximum temperatures were calculated to range from 1.7 °C to 15.3 °C (3.5 °C to 17.1 °C at Braemar); and mean daily minimum temperatures from -4.1 °C to 7 °C (-2.8 °C to 8.3 °C at Braemar). Snow lie was estimated to be about 140 d a year -

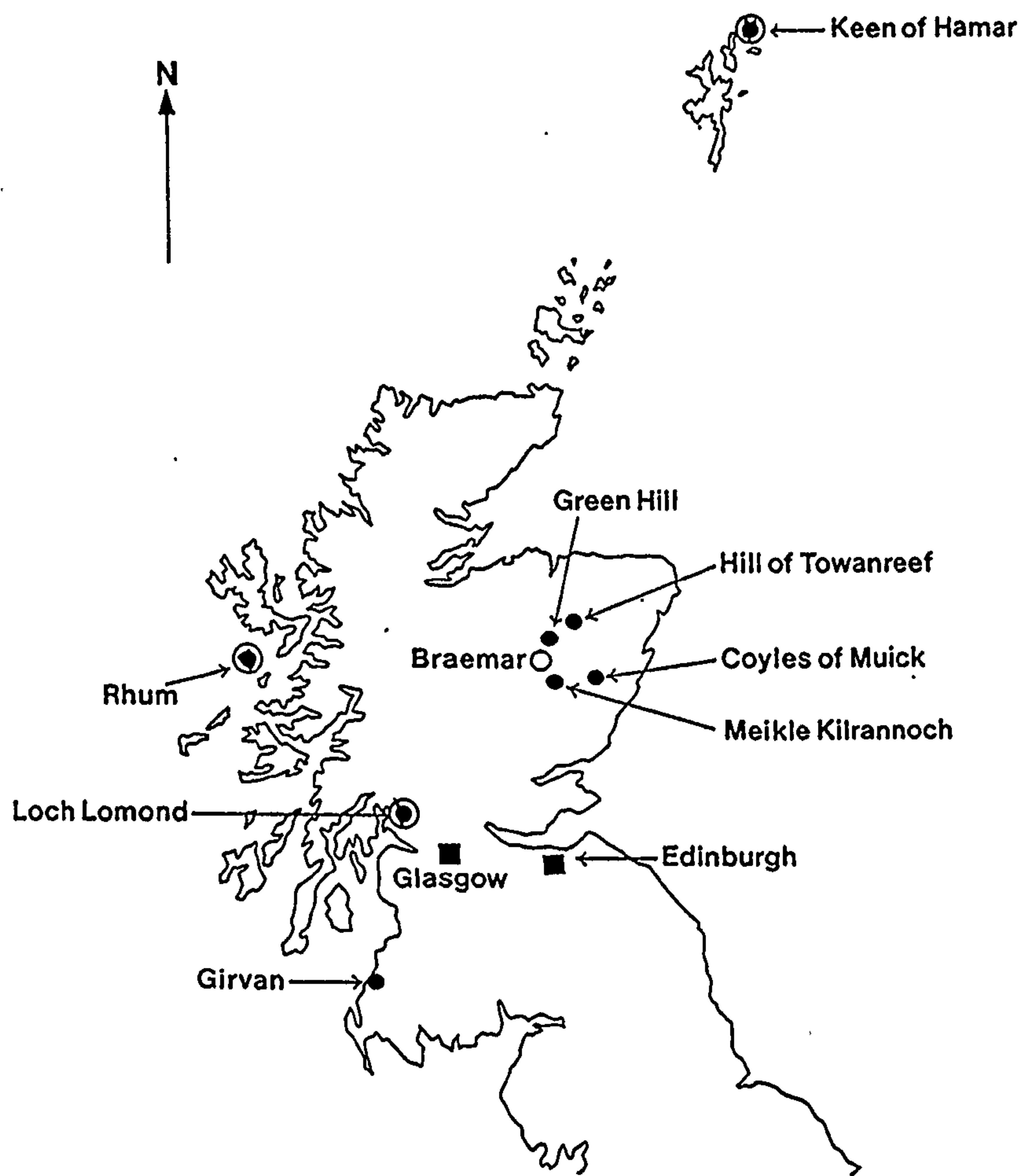


Fig 1.1 The location of the main Scottish ultramafic areas ●; and sites of their local meteorological stations ○ (from Proctor 1992).

between November and April. Actual snow lie at the ultramafic sites was found of much less duration between 1990-1994 (personal observation). Temperature was recorded continually at 1 cm below soil surface by a Grant temperature recorder (Grant Instruments) during the period 2-5 August 1993 and the minima and maxima ranged from 2 °C to 23 °C, the latter maximum on a sunny day.

Soil analyses at MK1, MK1.5 and MK2 have invariably shown high concentrations of exchangeable magnesium, low concentrations of calcium and high Mg/Ca quotient, and relatively high concentration of nickel.

The unusual floristics of the site (almost certainly MK1) were evident from the collections of G. Don in the late eighteenth century (Smith 1811). Marshall (1959) discussed the vegetation on the skeletal areas of MK2. The early brief descriptions of MK1 (Proctor 1969, Proctor & Woodell 1971, Johnston & Proctor 1980, Birse 1982) were superseded by a detailed description of the soils and vegetation of MK1 and MK2 (Proctor *et al.* 1991).

A major topic of ecological research on ultramafics has been the causes of ultramafic exclusion of non-ultramafic species and the tolerance and physiological requirements of ultramafic species. Bioassays demonstrated the extreme toxicity of the MK1 soil for non-adapted plants (Proctor 1971a,b). Later work used solution cultures based on soil solution extracts to test for magnesium and nickel toxicities in ultramafic and non-ultramafic races of Festuca rubra, and the role of calcium and micronutrients in ameliorating those toxicities (Johnston & Proctor 1981). The contrasting effects on the growth of Lychnis alpina of fertiliser addition to MK1 soil and peat in a growth room experiment have been reported (E. Brown & J. Proctor unpublished). More recent work investigated the tolerance of Armeria maritima from MK1, from a coastal habitat, and

from a lead mine spoil, to each other's soil (Goodwin-Bailey et al. 1992).

Previous work has confirmed the unusually toxic nature of the MK1 soil and has gone some way to showing plant adaptation^{to} it. More investigations were needed to render information about how the different species growing on the site responded to site characteristics. The present work focused on vegetation patterning, and soil-vegetation correlations, population dynamics of Lychnis alpina (one of the outstanding rarities at Meikle Kilrannoch) and on the site carrying capacity at MK1 and on species and overall vegetation responses to fertiliser addition at MK1.5.

The principal aims of the present studies were to address: the influence of habitat characteristics at different scales on the distribution of plant species on the skeletal areas of the site; to test experimentally, hypotheses on the causes of the unusual vegetation which were generated by the investigation of the habitat; to test the hypothesis that nutrients may limit growth on the skeletal soil by observing plant responses to added nutrients; and to rephrase the problems of the site's barrenness and its rarities in terms of site 'carrying capacity' (Taylor et al. 1991) and its components. Latin names of plants follow Stace (1991) with the exception of the genus Cochlearia the authorities for which are from Rich (1991)

Chapter II

Soil micro-spatial variation and vegetation patterning on the fellfield type skeletal soil at MK1. I. Variation in the soil environment and vegetation

Introduction

This chapter deals with the spatial patterning of the soil environment and vegetation. The next chapter deals with the correlations between them and the scale dependence of the pattern and the correlations.

Soil properties and vegetation usually display a pattern of some form (Greig-Smith 1979). The term 'pattern' for use in vegetation ecology was defined by Greig-Smith (1983) as a 'non-random distribution of species'. Kershaw & Looney (1985) classified pattern according to causal factors at three different levels: (a) the morphological level; (b) environmentally induced pattern, of one or more scales, of density distribution (β -diversity); and (c) 'derived pattern' - intrinsic properties of the species growing together and of microenvironmental variation.

The importance of soil resource availability for vegetation development processes has been a focus of community ecology (Grime 1979) and warrants the detailed study of spatial patterning of soil characteristics and vegetation. The importance of environmental heterogeneity in regulating community processes by influencing plant-to-

plant interactions has been stressed by Fowler (1988). The first step in tackling the relationships between the environment and vegetation is to show that nutrient availability does vary in characterisable fashion over the area under investigation. A large number of studies have been carried out using various methods to show that variation (pattern) in the environment causes variation in vegetation at the small to medium scale (Legendre & Fortin 1989).

High levels of variability in the soil environment have been found in earlier studies (e.g. Snaydon 1962). Over the past ten years an increasing number of publications have dealt with the methodology to overcome the deficiencies of characterising soils by the overall means of variables which blur locally important variation (Webster 1985, Trangmar et al. 1985). The use of regionalised variables (geostatistical methods) has been proposed to describe this variation which is not independent of space or time but follows a non-random pattern. There have been many reports recently on thus characterising the soil environment for a variety of ecosystems (e.g. Lechowicz & Bell 1991b, Palmer 1990, Robertson et al. 1988, Kelly & Canham 1992, Jackson & Caldwell 1993) in the wake of introductory discussions of the application of geostatistics in ecology by North American workers (Robertson 1987, Rossi et al. 1992). The common feature of these studies was that they used variograms to describe spatial dependence and directionality of soil properties. Variograms are half variance values at known separation distances plotted against those distance points and are calculated according to the following formula:

$$\gamma(\mathbf{h}) = 1/2N(\mathbf{h}) \sum_{(i,j) \text{ hij}=\mathbf{h}} (v_i - v_j)^2$$
 where γ is the semivariance, v_1, \dots, v_n are the data values summed over only the data pairs which are separated by \mathbf{h} distance, and N is the number of samples (Isaaks & Srivastara 1989).

The advantage of using variograms as opposed to traditional block-size techniques (e.g. Greig-Smith 1952) in detecting pattern in vegetation has recently been argued by Palmer (1988), who pointed out that variograms plot variance as a continuous function of scale, resulting therefore in a less angular diagram than the block-size method. Also, the scale limitation of the block-size method is overcome when variograms are used to describe pattern.

Previous work has established that the unusual floristic composition of the vegetation on skeletal soil at MK1 is at least partly due to soil chemistry (Proctor *et al.* 1991). Other types of soil at the site support fully developed low-alpine grass heath, and in some low-lying patches, blanket bog. No study had been made of the microspatial environmental variation in habitat characteristics on the open areas of MK1 and how different conditions there might affect plant growth.

The aims of the present study were: (1) to characterise soil spatial variation at two scales (0 - 300 cm and 0 - 900 cm) and to describe spatial structure; (2) to detect the patterning of individual species and the assemblage of species as a whole by using variograms.

Material and Methods

STUDY SITE

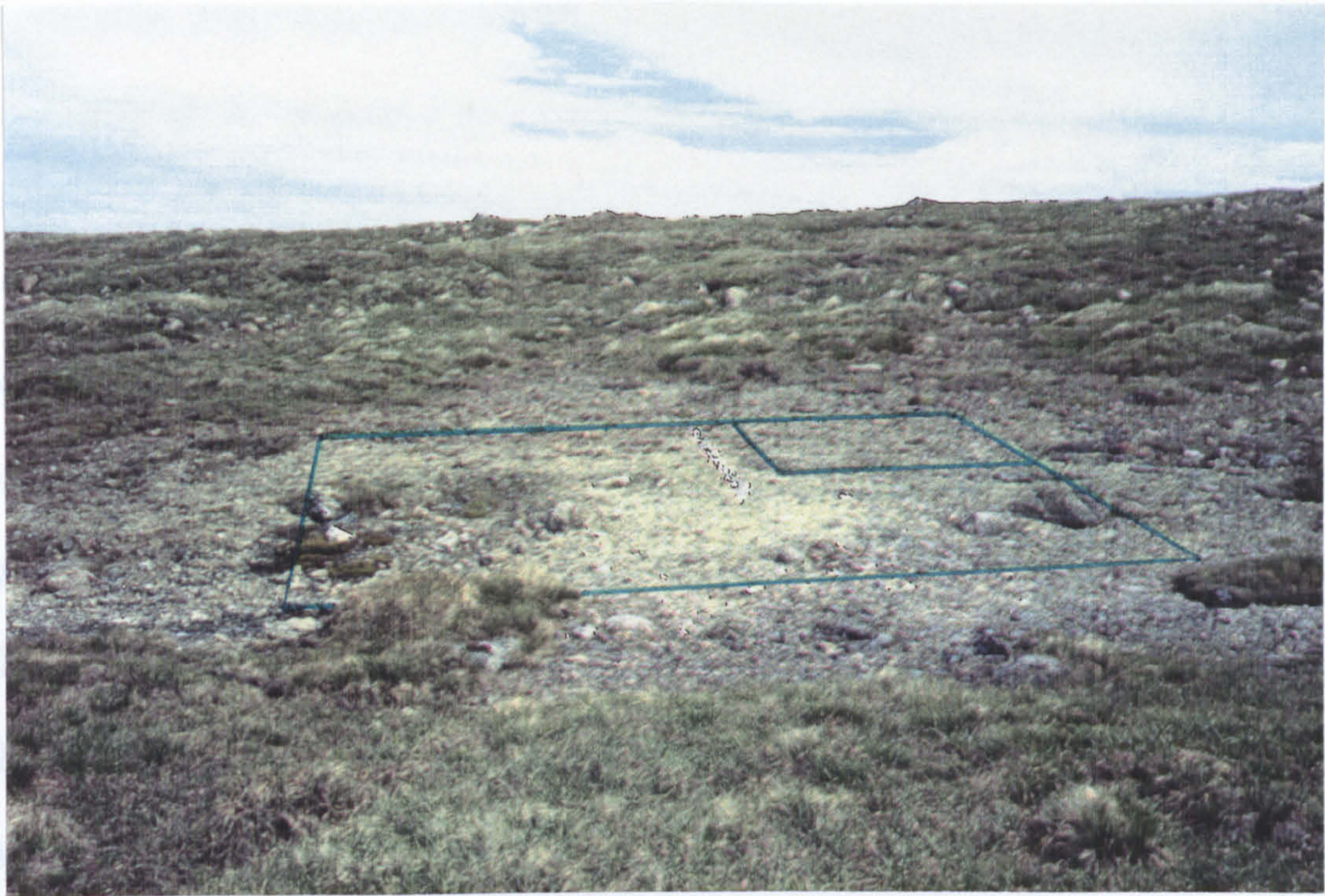
The study was carried out at the south-west tip of MK1 where a sample area of 3 m x 3 m (later extended to 9 m x 7 m) was chosen for intensive investigation. The area faced west and was at the bottom of a short slope of about 9° (Fig. 2.1). The area was

chosen because it showed a gradient in plant distribution along its slope and included a part which was completely devoid of vegetation and it was thought that environment - vegetation correlations would be readily identified there. The area had a skeletal soil with sparse vegetation restricted to a few species, mainly Agrostis vinealis, Armeria maritima, Cerastium fontanum ssp. scoticum, Cochlearia pyrenaica ssp. alpina, Festuca rubra and Lychnis alpina. The vegetation immediately up-slope of the area was short alpine grass heath - an intermediate form between the sub-communities Empetrum nigrum ssp. hermaphroditum - Cetraria islandica and the typical sub-community of the Nardus stricta - Carex bigelowii community (Rodwell 1992). There was exposed bedrock at the top of the slope above the short alpine grass heath.

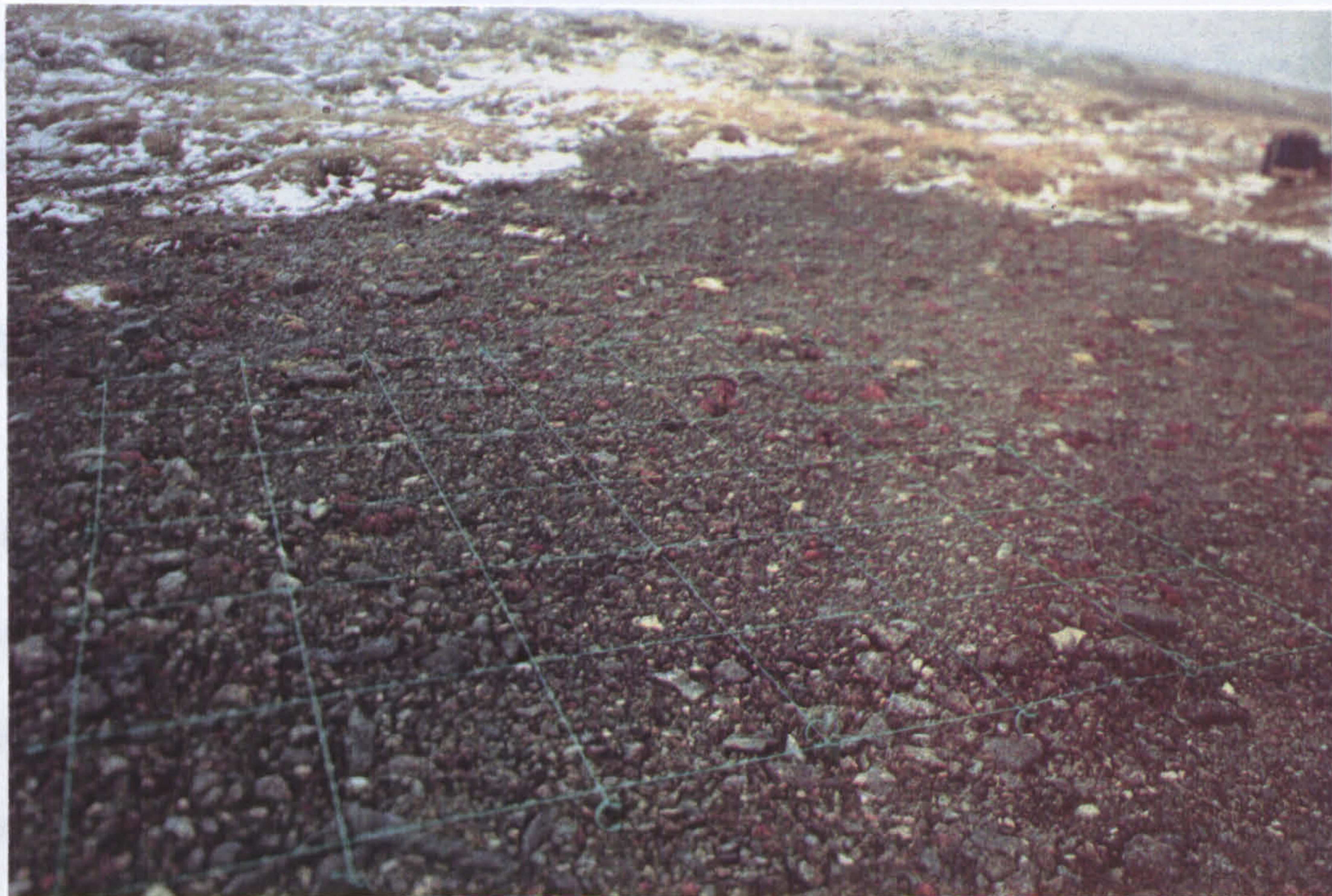
SAMPLING AND ANALYSES

Sampling design

The sampling was designed to produce samples for the investigation of the spatial dependence of soil characteristics at two different scales (Fig. 2.1a,b). The 3 m x 3 m area was spatially regularly sampled between 8-15 May 1992 following Webster's (1985) recommendations. Two hundred and twenty-eight samples were collected, thirty-six of which were at the intersects of a grid of 50 cm x 50 cm overlying the 3 m x 3 m area. The remaining 192 samples were grouped in eight clusters each of twenty-four 10 cm x 10 cm quadrats in two fixed (Nos. 1 and 36) and six randomly selected (Nos. 3, 7, 10, 18, 26 and 34) 50 cm x 50 cm sub-plots (Fig 2.1b). The sample area was extended to 9 m x 7 m at the second sampling between 28 September and 3 October



(a)



(b)

Fig 2.1 The area used for the pattern study described in the text. The upper photograph (a) shows the total 9 m x 7 m area sampled in October 1992; the lower photograph (b) shows in more detail the 3 m x 3 m area (from the top right hand corner of the area of the upper photograph) which was sampled in May 1992.

1992 (Fig. 2.1a). Samples were taken from thirty-six points at the 50 cm x 50 cm intersects of the original 3 m x 3 m grid. Further samples were collected from the intersects and the middle of the squares of a 100 cm x 100 cm grid within the 9 m x 7 m sample area. The October sampling had 144 samples. For logistical reasons, samples for soil chemical characters were not collected at the fine (10 cm x 10 cm) scale in October. However, vegetation data were recorded for an additional six 50 cm x 50 cm sub-plots (Nos. 37, 50, 59, 67, 84 and 88) at that scale, resulting in an extra 144 samples. It is of importance for constructing variograms that the number of samples is sufficient. Webster (1985) recommended that for transects the number should be about one hundred and at least eighty pairs should be available for comparison at each lag distance, although more conservative estimates set these figures higher. The numbers of samples on both sampling occasions were sufficient to produce reliable estimates of spatial dependence for the variables investigated as the number of pairs at each lag was at least five hundred.

Vegetation

The density of all plants was recorded in the 10 cm x 10 cm quadrats. Two hundred and twenty-eight samples were collected in May and 288 (144 + 144) in October. For graminoids the number of ramets was recorded, while species with distinguishable genets (e.g. Armeria and Lychnis), were recorded as single plants irrespective of the number of rosettes.

Soil chemistry

Ion-exchange resin bags were used to assess nutrient availability. Sibbesen (1978) and Smith (1979) have argued that ion-exchange resins can be used as model roots because their ion uptake capacity character is similar to that of plant roots. A number of authors have reported that results for ion-exchange resins reflected nutrient availability better than traditional methods (Tran et al. 1992, Smith 1979, Cooke & Hislop 1963). By using ion-exchange resin bags it was thought that a more realistic estimate could be obtained for nutrient availability as this is an in situ method which also allows for the effect of varying soil water.

The resin bags were prepared according to Gibson et al. (1987). Nylon mesh with an average aperture size of 400 μm (Plastok Associates) was used to construct bags of 7 cm x 7 cm. Seven g of cation exchange resin (Amberlite IR 120(plus), supplied by Aldrich Chemical Co. Ltd.) and 5 g of anion resin (Amberlite IRA-400(Cl)) were weighed out and mixed in each bag. The bags were heat-sealed and regenerated by shaking for 30 min with each of three changes of 5% hydrochloric acid and then rinsed in deionised water. Following the recommendations of Proctor & Nagy (1992) for sampling depth on ultramafics, the bags were placed about 5 cm below the soil surface and recovered after 5 d. (In a pilot study carried out in the laboratory, resin bags were left in MK1 soil with different water contents (moist and field capacity) for 1 d and 5 d. It was found that the 5 d period was appropriate to reflect differences in ion availability in soils of both water contents without the risk of exceeding the exchange capacity of the resins.) Care was taken to minimise disturbance to the structure of the soil at the sampling points. A trowel was used to lift up the upper 5 cm of soil in one piece and after placing the bags under it, a slight tapping ensured good contact without compaction between the soil and the bags.

The ion-exchange resin bags were sealed in plastic bags individually at collection and were kept in a cold room at 4 °C until processing. The bags were washed in running deionised water to remove soil attached to their surface and then were eluted in 100 ml 5% HCl in an end-to-end shaker for 30 min. The eluates were filtered through Whatman No. 44 filter papers and stored in polythene bottles.

Potassium, calcium, magnesium, iron and nickel concentrations were determined using a Varian AA-575 atomic absorption spectrophotometer. Phosphorus for the May samples was measured colorimetrically after a two-fold dilution of the samples on a Fiastar 5010 flow injection auto-analyser using the stannous chloride-ammonium molybdate method (Fiastar Application Sheet No. 60-02/83). Phosphorus was also determined using the ammonium molybdate - potassium stannous tartarate method. Nitrate-nitrogen was determined colorimetrically by using the sodium salicylate method and ammonia-nitrogen by titration with sodium hypobromide after neutralising with sodium hydroxide.

For pH measurements, soil cores were collected from the intersects of the 50 cm x 50 cm grid and also from three sub-plots of twenty-five quadrats each in early May 1992. The sampling points were different from those for the available-ion determinations to avoid interferences caused by exchanged protons and chloride ions and also to allow further sampling for nutrients at a later date. Measurements of pH were made on 10 g of fresh soil mixed with 25 ml 1M KCl at room temperature.

Physical characters

Angular stones cover much of the soil surface of the area investigated. To assess stoniness, photographs of the 10 cm x 10 cm quadrats were visually scored on a 1 to 5 scale (1, quadrat surface dominated by one single stone; 2, one large piece of stone taking up half of the quadrat; 3, one piece covering up to 25% of the surface, rest is rough grained; 4, medium grained, homogenous or heterogenous; and 5, fine grained, homogenous; see Fig. 2.2). These values 1-5 were converted to plant available-area values calculated as described below. The simplified assumption was made that the surface stones are two dimensional and that as they are cut successively into four of diminishing sizes the cracks or generated lines (which would be available for plant colonisation) follow a mathematical sequence. If the area overlain by a quadrat, with sides of unit length, is of a one-piece solid rock which is bigger than the quadrat, then that area is not available for plant growth and a value of zero is assigned to it. In the case where the stone is just smaller than the quadrat an 'available-area' of four unit lengths is available for plant colonisation. If this is divided into four (which would represent stone sizes of 5 cm x 5 cm) the total 'available-area' would be six, and further division would result in ten. Thus fragmentation (F) would follow the formula: $F = 4u + 2^n - 2$, where u is unit length of the side of the experimental quadrat and the n is the number of division into four of the previous stone size. The fragmenting theoretical stone would therefore provide an increasingly higher 'area' for plant colonisation (Fig. 2.2)

Soil depth was estimated in the 144 quadrats sampled by resin bags at the October sampling. A graduated metal rod was forced into the soil until it hit bedrock, or any larger stone. To avoid spurious estimates arising from hitting larger stones rather than true bedrock, five values were obtained for each quadrat of which the extremes were

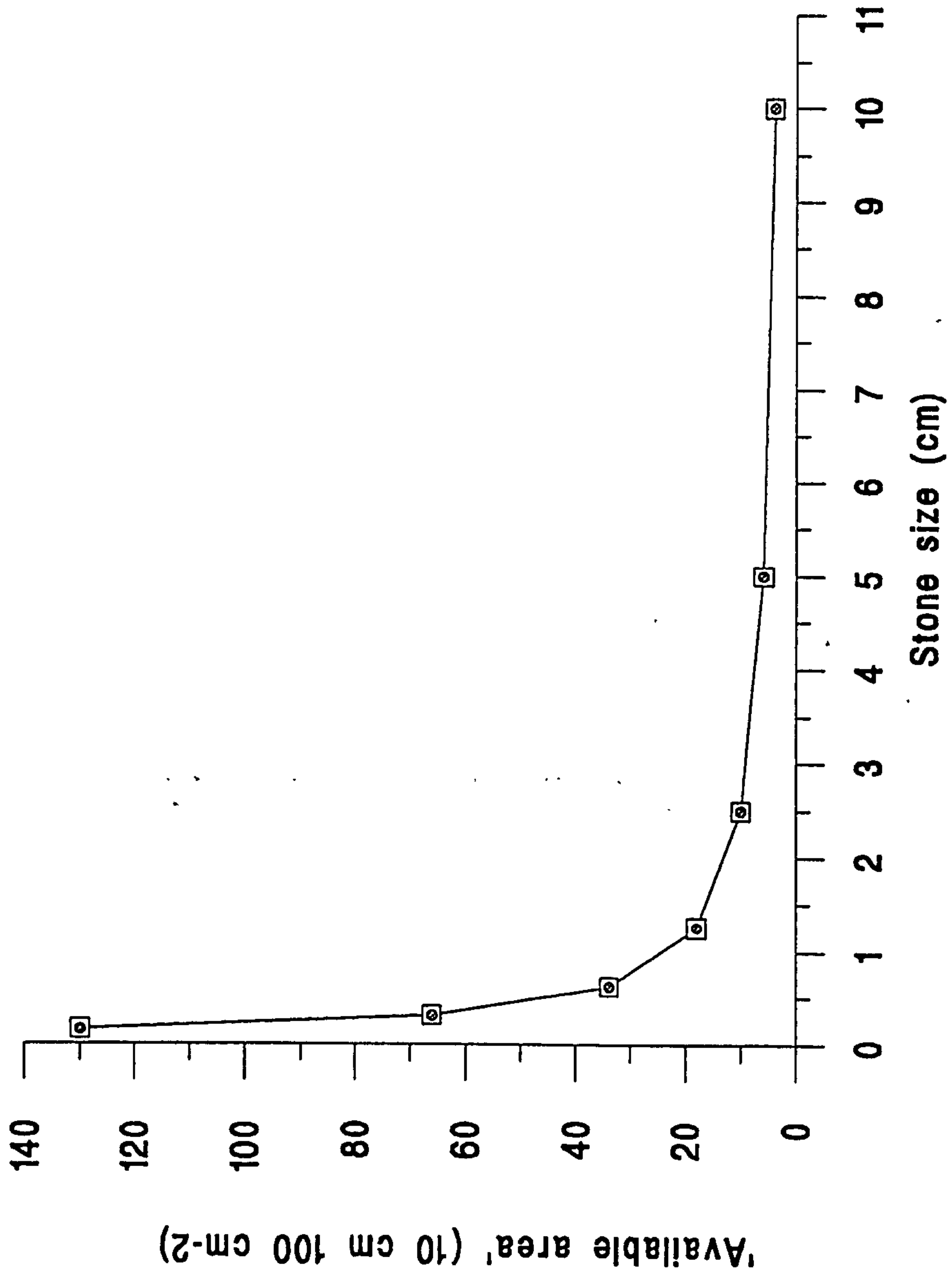


Fig 2.2 Relationship between stone size and 'available area'. For explanation see text.

disregarded in the calculation of mean soil depth.

Slope measurements were made to yield information about the position of the quadrats along the slope relative to the highest point. The distances from a horizontal plane projected from the highest point were calculated for the intersects of the 1 m x 1 m grid by taking readings of the distances at those points in two directions along the two main axes of the area.

Spatial dependence (pattern)

Geostatistical methods were used to study spatial structure at different scales (0-300 cm and 50-900 cm) using the software package Geopack v1.0e (Yates 1990). Variograms were constructed for the soil variables investigated to describe spatial dependence by illustrating the average degree of similarity between semivariance values of each variable as a function of sampling separation distance. The interpretation of spatial dependence by variograms is based on the fact that spatial (and also temporal) variables are more likely to change gradually over distance (or time) rather than abruptly. This will be indicated by increasing variance values up to a so-called 'sill', after which the variance equals the overall sample variance (mean variance). Samples of spatial variables separated by distances less than the range (the distance between zero distance and the distance at which the sill is reached) are spatially (or temporally) dependent, and therefore predictable from known values. The range is a measure of spatial dependence. In some cases the variances of soil properties appear to have no limit, that is they show no finite variance and do not reach a sill (unbounded variograms). In other cases there may be a complete absence of spatial structure implying the lack of

quantifiable spatial relationships at the scale used.

The sample variograms were used to create model variograms by least-square fitting techniques for the 'kriging' routine (an estimation method based on reciprocal averaging and taking into account spatial dependence and directionality) to estimate values for constructing maps with different resolutions for the study area. Most variograms generally approximate simple forms which are amenable to fitting simple functions to them. There are four main types of model which are most frequently used: linear models, spherical models, exponential models and hyperbolas (Webster 1985). Not all variogram curves will go through the origin of the co-ordinate axes but will indicate some degree of variability at distance zero ($h_1=0$). This so called 'nugget effect' (Journel & Huijbregts 1978) may be caused by intrinsic random variation in the variables or it may suggest that spatial dependence is not detectable at the scale used. Block diagrams were used to display the patterns of variation for the variables investigated. In the gradient analysis (Chapter III) the values for environmental variables at the 20 cm x 20 cm, 30 cm x 30 cm, 40 cm x 40 cm and 50 cm x 50 cm spatial scales were derived from maps constructed by the above method for the six 50 cm x 50 cm quadrats sampled in October.

Variograms were constructed to detect pattern for each of the main plant species occurring at the site using a combined total of 480 vegetation data available from the two samplings (228 + 288 - 36 for overlapping samples = 480). The density scores were log-transformed before analyses to reduce the weighting of occasional high values.

Variograms were also constructed using the total information of the vegetation samples. To be able to assign a single value to each sample, principal components analysis (PCA) was done on the combined total of 480 samples and the sample scores for the

first axis were then used in calculating the variograms. After an initial log-transformation no further transformation was done on the data matrix, thus the derived values on the first PCA axis were all positive. High numbers of quadrats contained no plants at all. These non-occurrences are usually not considered in ordination. However, since in this case the aim of the PCA was to summarise the vegetation data for each quadrat as a single value for the calculation of spatial dependence, quadrats with no plants were assigned a value of 0.1.

Results

SOIL CHARACTERISTICS

The pilot study and data from elsewhere (Gibson *et al.* 1987) showed that the amount of ions exchanged from the soil depends on the length of time for which the bags are left in the soil and on the amount of resin(s) used. Also, the pilot study showed the importance of the water content of the soil for ion availability. The same protocol was followed at all sampling points at both times which allowed comparisons of ion availability in space and time. It should, however, be pointed out that comparison of the results with those obtained by other methods has to be done with caution.

Summary statistics for the soil analyses of the May and October sampling and measurements on microtopography are presented in Tables 2.1 and 2.2. Mean values for NO_3^- , K^+ , Ca^{2+} , Mg^{2+} , iron (the ionic species were not determined and 'iron' will be used throughout in this chapter) and Ni^{2+} showed similar values both in May and October. Apparently more H_2PO_4^- was exchanged by the resins in October, although this

	NO ₃ ⁻ (mg l ⁻¹)	H ₂ PO ₄ ⁻ (mg l ⁻¹)	K ⁺ (meq)	Ca ²⁺ (meq)	Mg ²⁺ (meq)	Iron (mg l ⁻¹)	Ni ²⁺ (meq)	Available area	pH _(KCl)
No. of samples	190	222	227	227	227	219	227	202	91
Mean	7.9	0.28	0.13	1.28	27.0	28.8	0.23	14.4	5.55
Median	5.3	0.21	0.10	1.2	25.0	23.7	0.19	17.0	5.60
Stdev.	0.7	0.01	0.004	0.04	0.91	1.32	0.007	0.26	0.38
Variance	92.0	0.045	0.003	0.37	189	379	0.01	13.5	0.14
Skewness	2.4	2.4	1.4	0.7	0.9	1.35	1.05	-0.76	-0.87
Kurtosis	10.25	11.8	5.0	3.1	3.8	4.8	3.95	2.5	3.0
Range	0.0-58	0.0-1.65	0.03-0.44	0.35-3.5	6.9-81	2.8-98	0.04-0.65	4.0-18.0	4.5-6.2

Table 2.1 Summary of the soil variables investigated at the 8-15 May 1992 sampling. The samples were collected using ion-exchange resin bags and the concentrations refer to concentrations of the ions in 100 ml 5% hydrochloric-acid eluate; available area is expressed in relative units as explained in the text.

	NO ₃ ⁻ (mg l ⁻¹)	H ₂ PO ₄ ⁻ (mg l ⁻¹)	K ⁺ (meq)	Ca ²⁺ (meq)	Mg ²⁺ (meq)	Iron (mg l ⁻¹)	Ni ²⁺ (meq)	Soil depth (cm)
No. of samples	139	144	144	144	139	132	144	142
Mean	7.16	1.50	0.12	1.40	32.84	33.47	0.20	16.11
Median	2.14	1.09	0.10	0.61	26.13	23.79	0.18	15.85
Stdev.	0.97	0.11	0.005	0.17	1.60	2.24	0.0075	0.39
Variance	129.96	1.76	0.004	4.03	351.53	658.33	0.009	21.05
Skewness	3.41	1.69	1.32	2.25	1.77	0.82	2.30	0.18
Kurtosis	17.75	5.84	4.69	7.72	5.55	2.62	10.53	2.18
Range	0.71 - 72.32	0.00 - 6.94	0.02 - 0.35	0.01 - 9.47	9.16 - 99.11	0.00 - 97.09	0.07 - 0.68	5.60 - 25.30

Table 2.2 Summary of the soil variables investigated at the 28 September and 3 October 1992 sampling. The samples were collected using ion-exchange resin bags and the concentrations refer to concentrations of the ions in 100 ml 5% hydrochloric-acid eluate.

may be due to the use of a different method for phosphorus determination for those samples.

VARIOGRAMS

Variograms for the environmental variables are shown in Figs. 2.3-2.7 separately for the May and October samplings. Summary tables for the model variograms for environmental variables and vegetation are given in Tables 2.3 and 2.4. A differing extent of spatial dependence was exhibited by the variables. NO_3^- data were not amenable to model fitting. They had a negligible level of autocorrelation with a range of about 75 cm followed by a stable level of variance up to about 1.5 m (Fig. 2.3). On the larger scale NO_3^- values were random (Fig. 2.6). H_2PO_4^- showed weak autocorrelation up to about 2 m then its variance remained stable (Figs. 2.3 and 2.6). Values at the fine scale for K^+ look erratic after 60 cm of spatial dependence because of the 'holes' at 1 m and 2.5 m (Fig. 2.3). These may reflect spatial periodicity of some sort (Trangmar *et al.* 1985). The variogram for the October data shows a clear spatial dependence with a range of 300 cm (Fig. 2.6), which is comparable with that obtained by Lechowicz & Bell (1992) in their study in a natural woodland. The variogram for Ca^{2+} consists of two phases, one with a sill at 75 cm, followed by another one, increasing linearly (Fig. 2.3). On the larger scale a weak spatial dependence can be seen to about 7 m (Fig. 2.6). Mg^{2+} on the small-scale had increasing variances to 130 cm followed by a linear phase as for Ca^{2+} (Fig. 2.4); on the larger scale, variance increased up to 4 m (Fig. 2.7). The strongest level of spatial dependence at the small-scale is shown by iron up to 75 cm (Fig. 2.4); on the larger scale, spatial dependence is clear

Soil variable	Scale (cm)	Range (cm)	Spatial dependence (%)	Model
Nitrate	0-300	-	-	n.a.
	0-900	-	-	n.a.
Phosphate	0-900	200	49	Spherical
Potassium	0-300	60	20 (estimated)	n.a.
	0-900	300	26	Gaussian
Calcium	0-300	75*	41	Spherical
	0-900	700	22	Spherical
Magnesium	0-300	130*	16	Spherical
	0-900	400	29	Gaussian
Iron	0-300	75	71	Spherical
	0-900	390	44	Spherical
Nickel	0-300	-	-	Linear
Stoniness	0-300	40	50	Spherical
Soil depth	0-900	390	87	Spherical

Table 2.3 Summary of the parameter values of the model variograms for soil chemical and physical variables shown in Figs. 2.3 - 2.7. Spatial dependence is calculated as $(C - C_0)/C$ where C is the value for the sill and C_0 the nugget. The lack of spatial dependence is indicated by n.a. for models; data are not presented for soil properties with apparent random spatial structure. * - initial flat phase of variograms.

Species	Scale (cm)	Range (cm)	Spatial dependence (%)	Model
<u>Agrostis vinealis</u>	0-300	40	48	Spherical
<u>Armeria maritima</u>	0-300	70	36	Spherical
<u>Cerastium fontanum</u> ssp. <u>scoticum</u>	0-300	60	52	Linear
<u>Cochlearia pyrenaica</u> ssp. <u>alpina</u>	0-300	-	-	n.a.*
<u>Festuca rubra</u>	0-300	40	50	Spherical
<u>Lychnis alpina</u>	0-300	40	46	Linear

Table 2.4 Model parameters for the variograms of plant species for the 0-300 cm scale.
* - no spatial structure was observed.

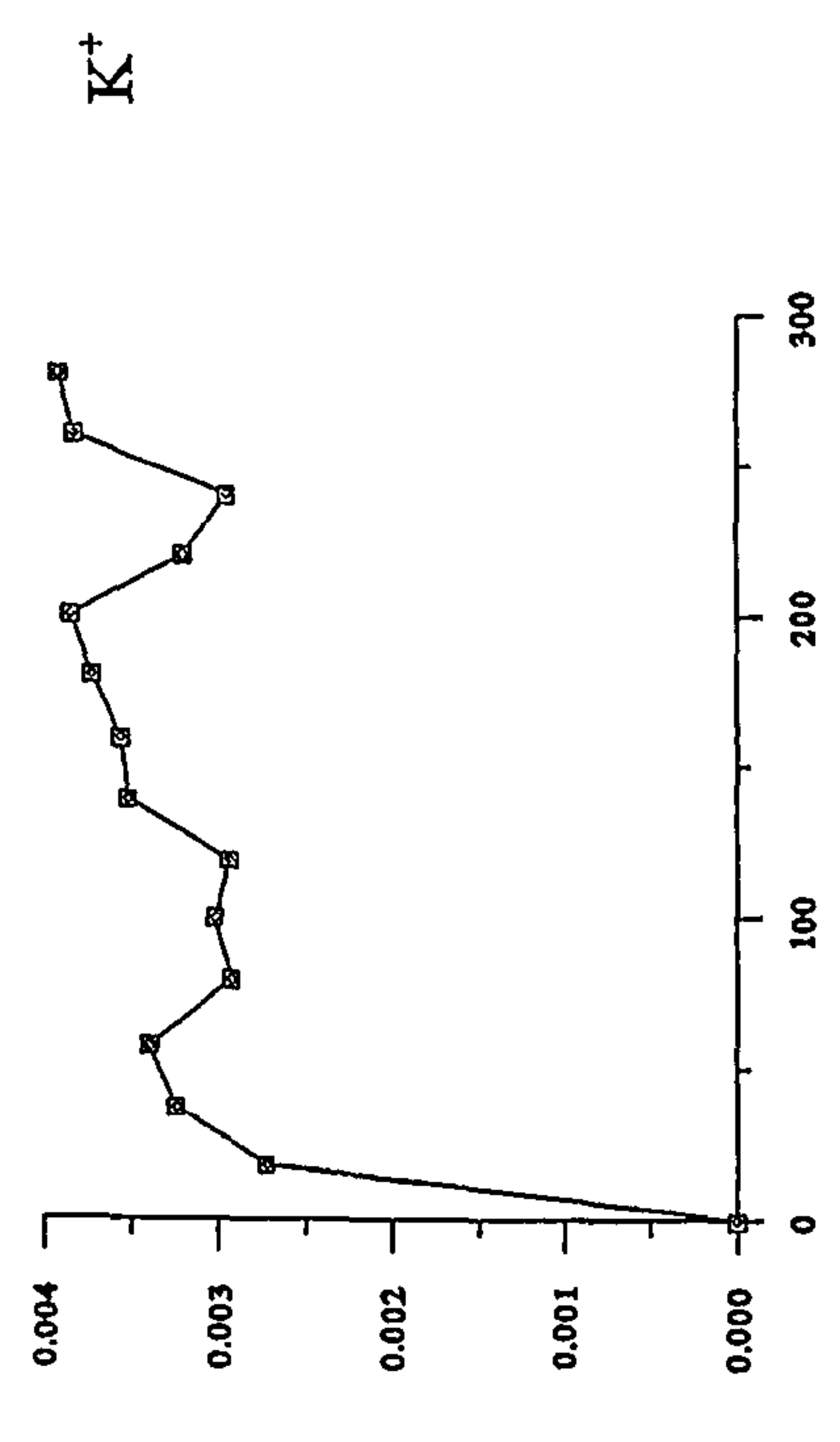
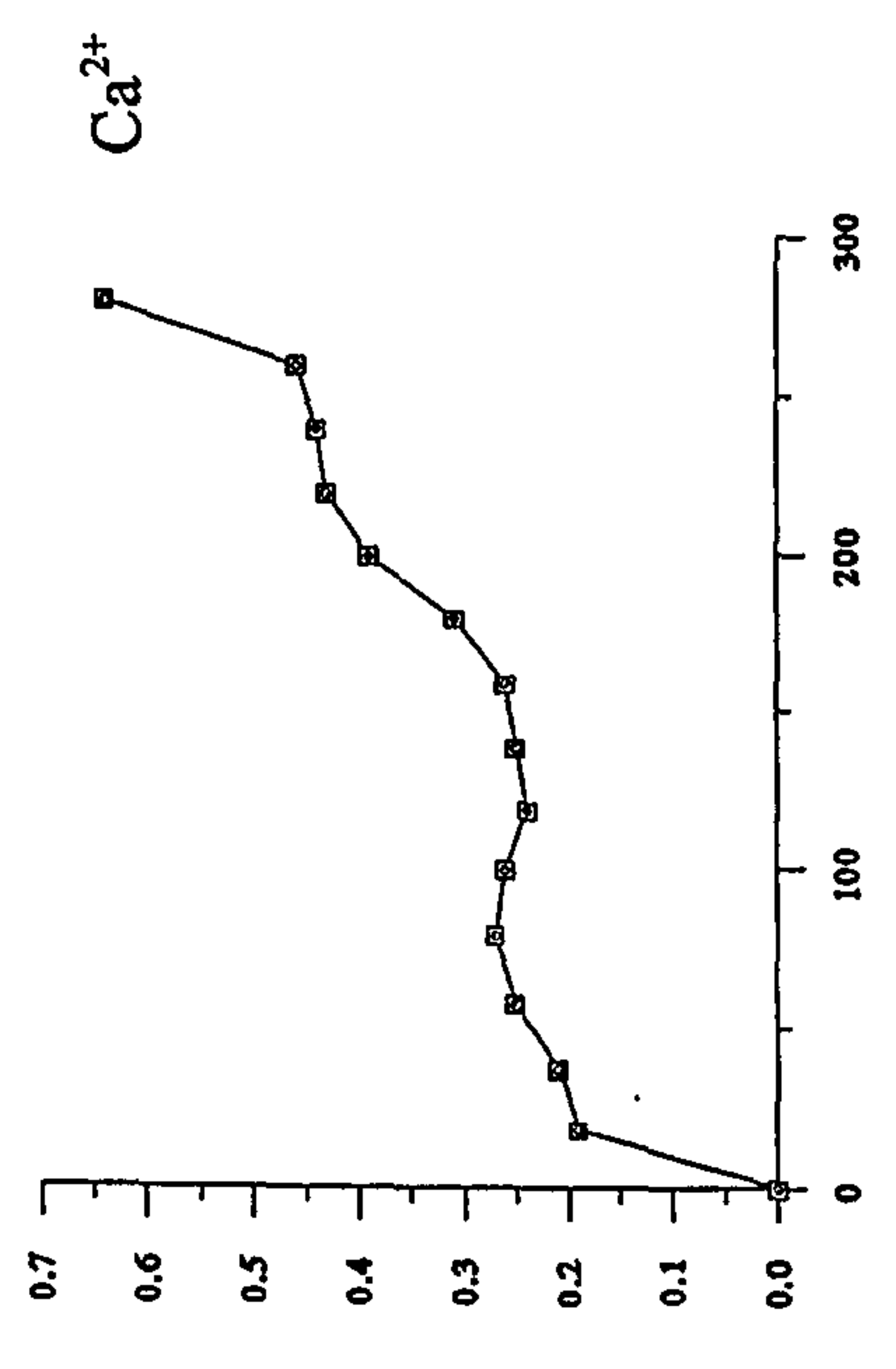
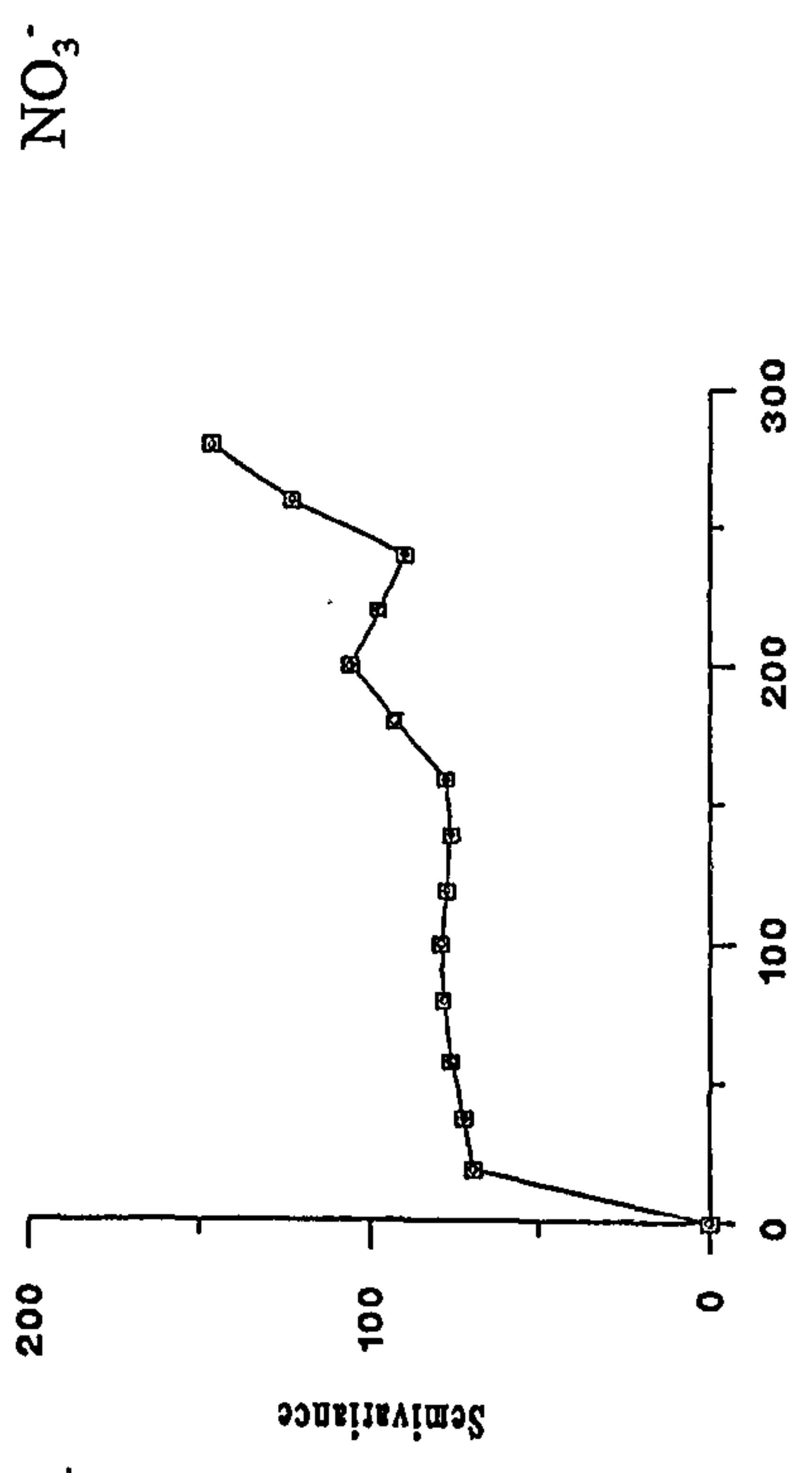
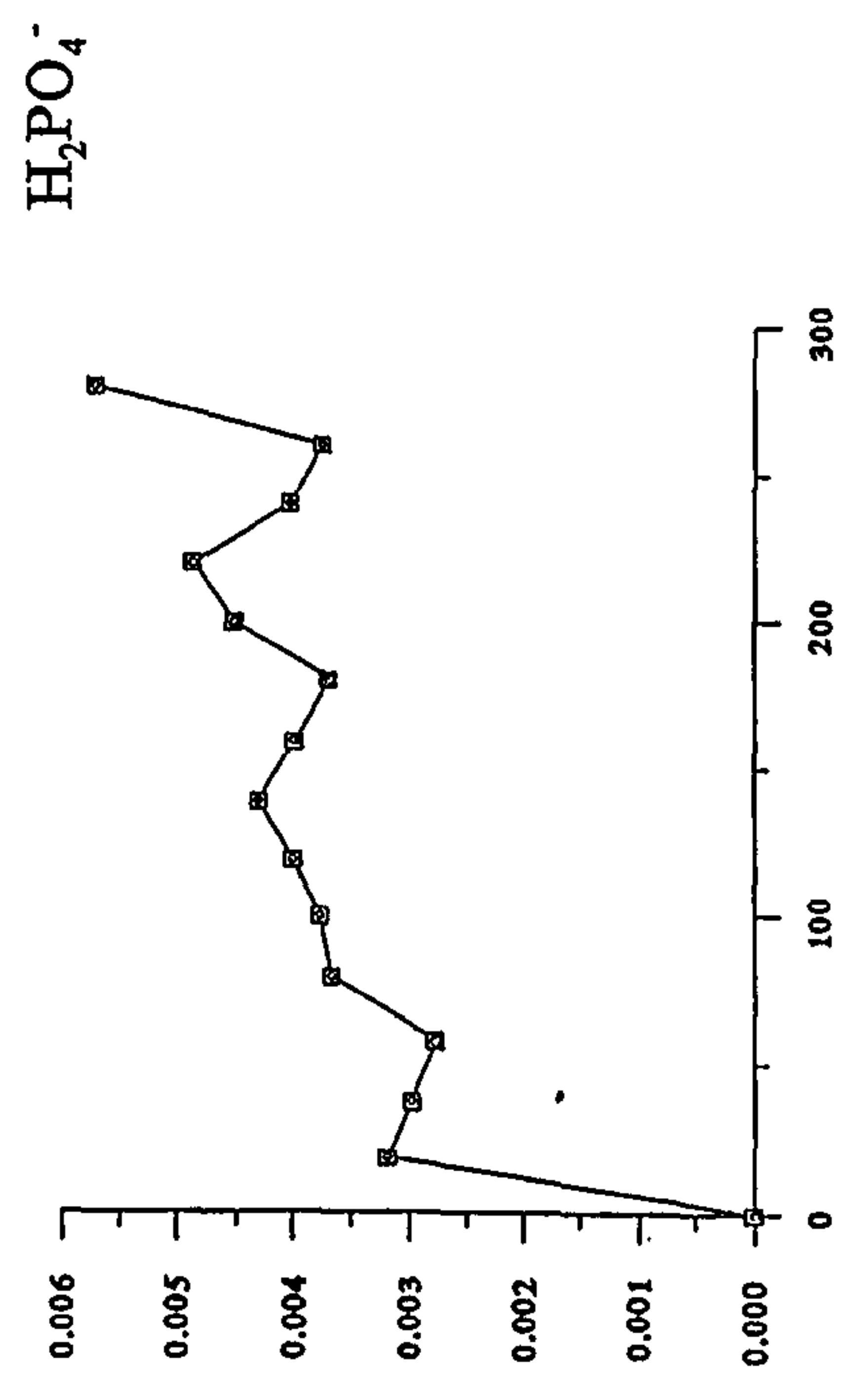


Fig 2.3 Variograms of soil NO₃⁻, H₂PO₄⁻, K⁺ and Ca²⁺ concentrations constructed from the 228 samples collected in May 1992. Values on the horizontal axes of Figs 3-11 are distances (cm); semivariance values are square units of attributes shown.

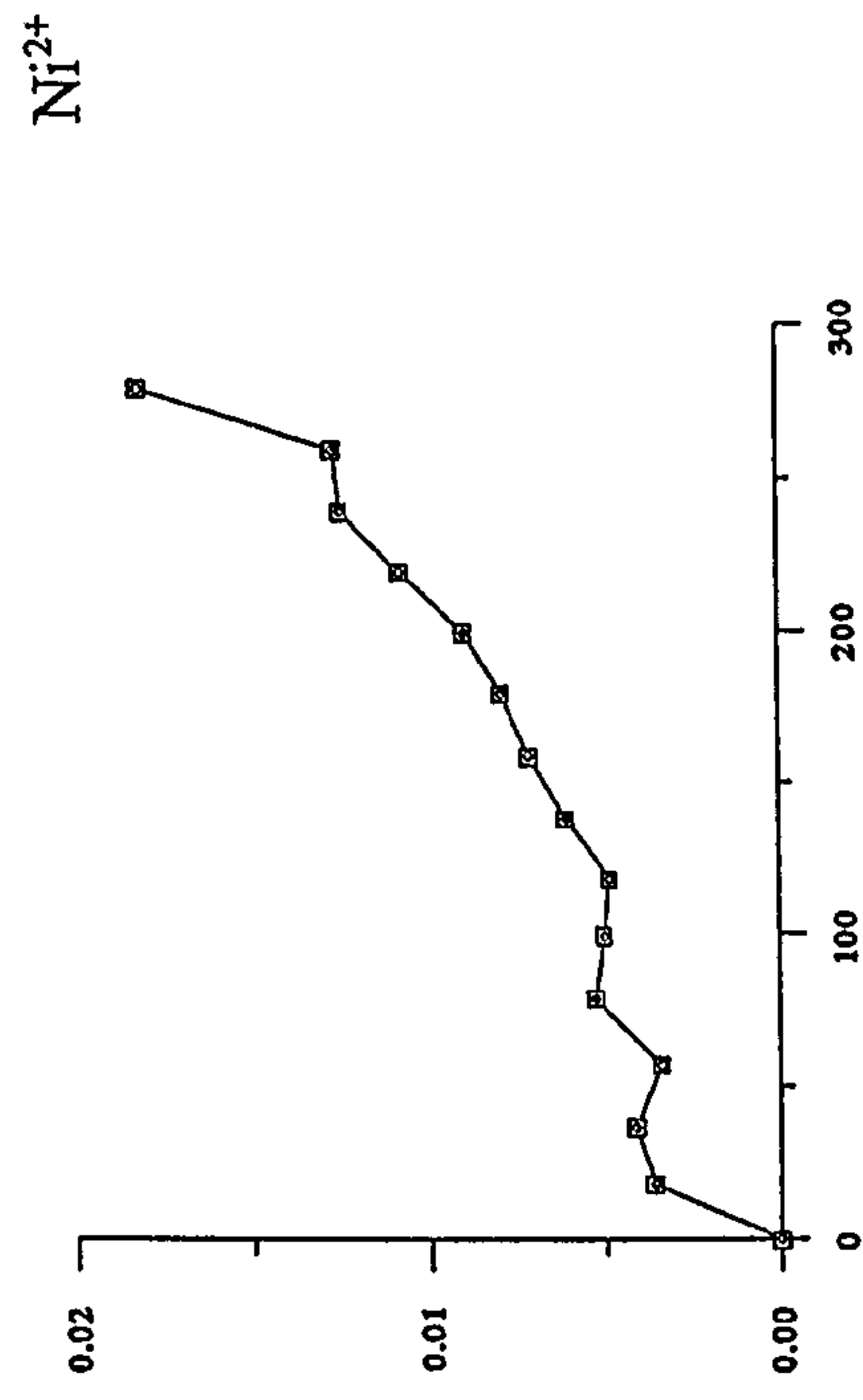
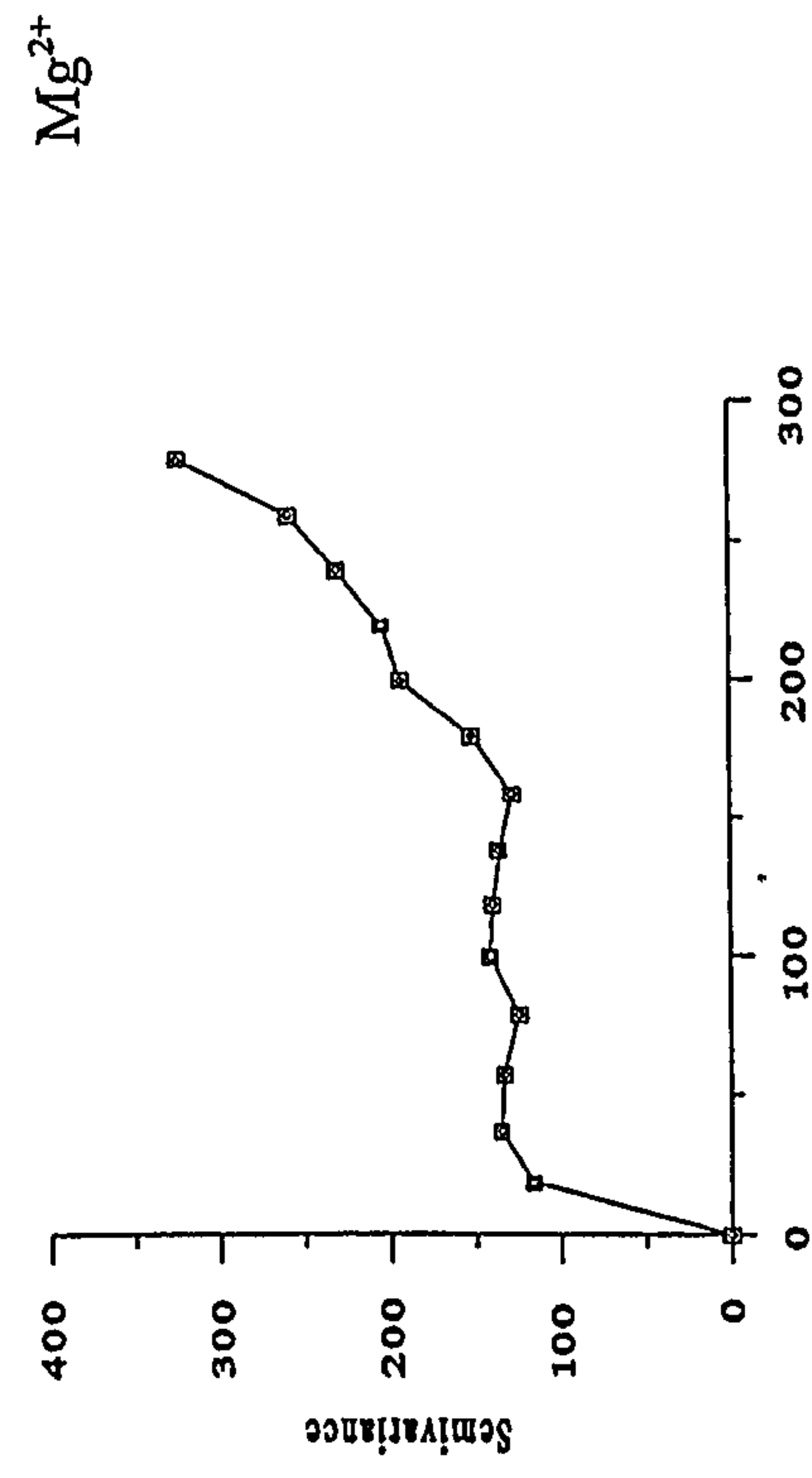
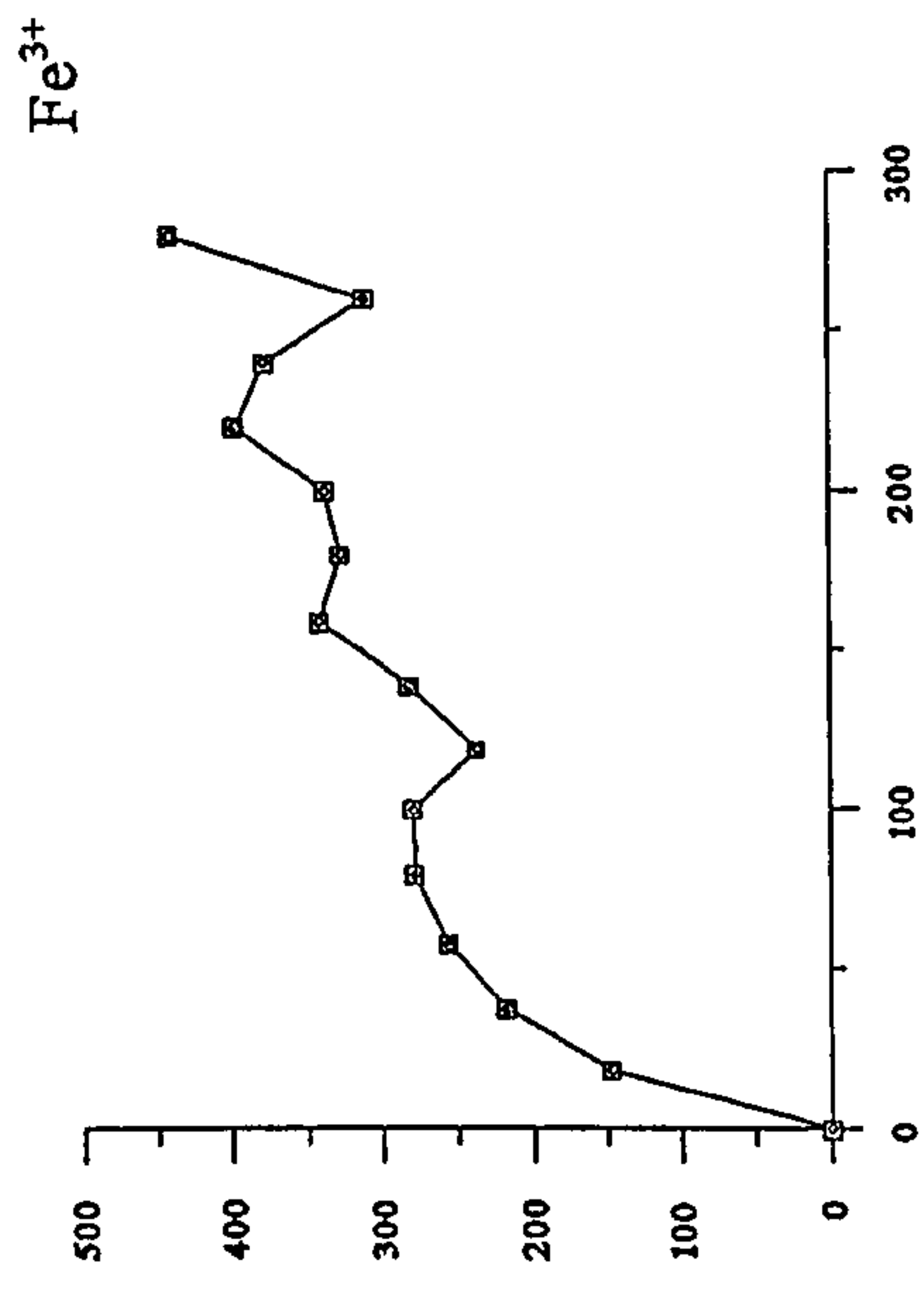
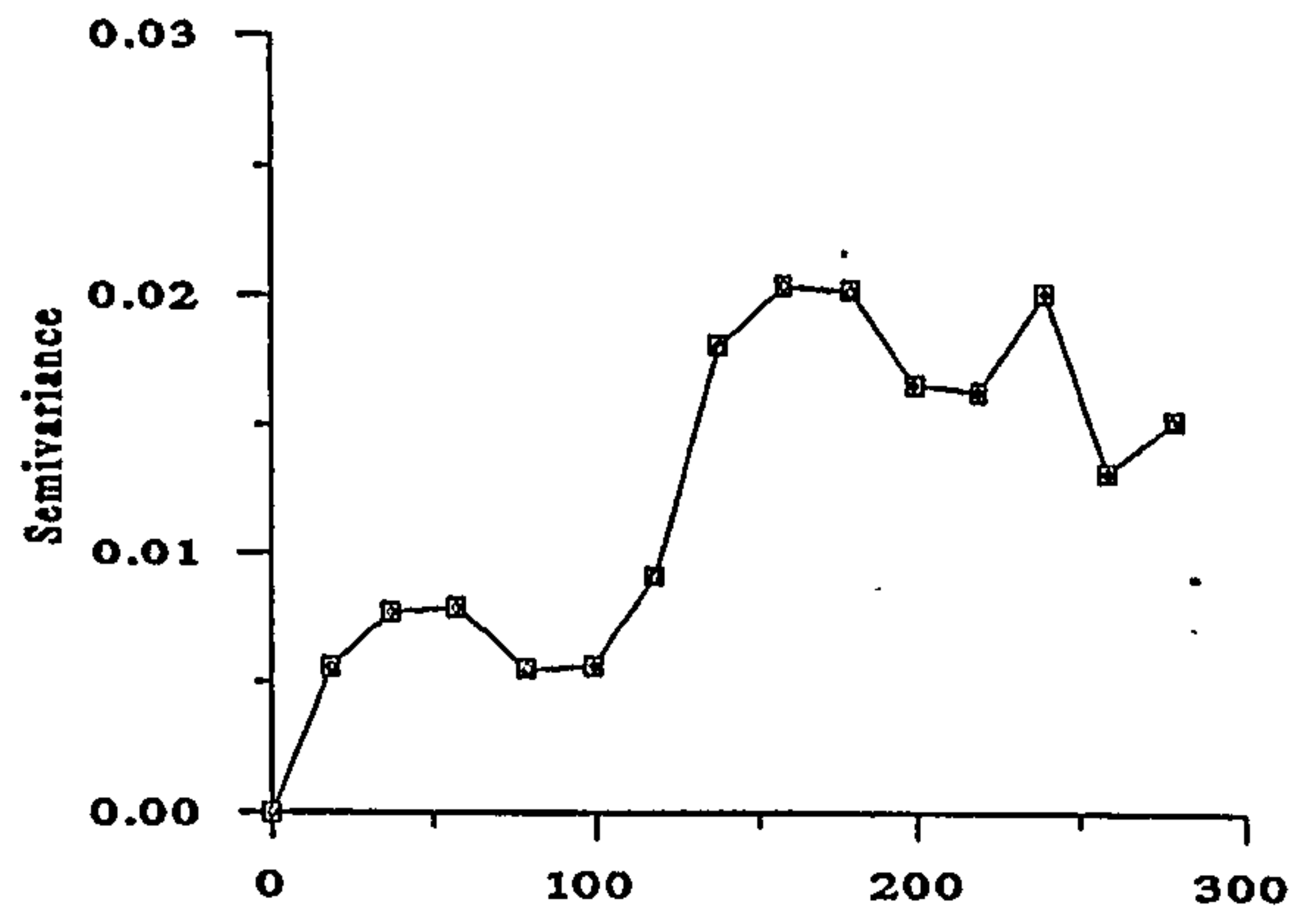
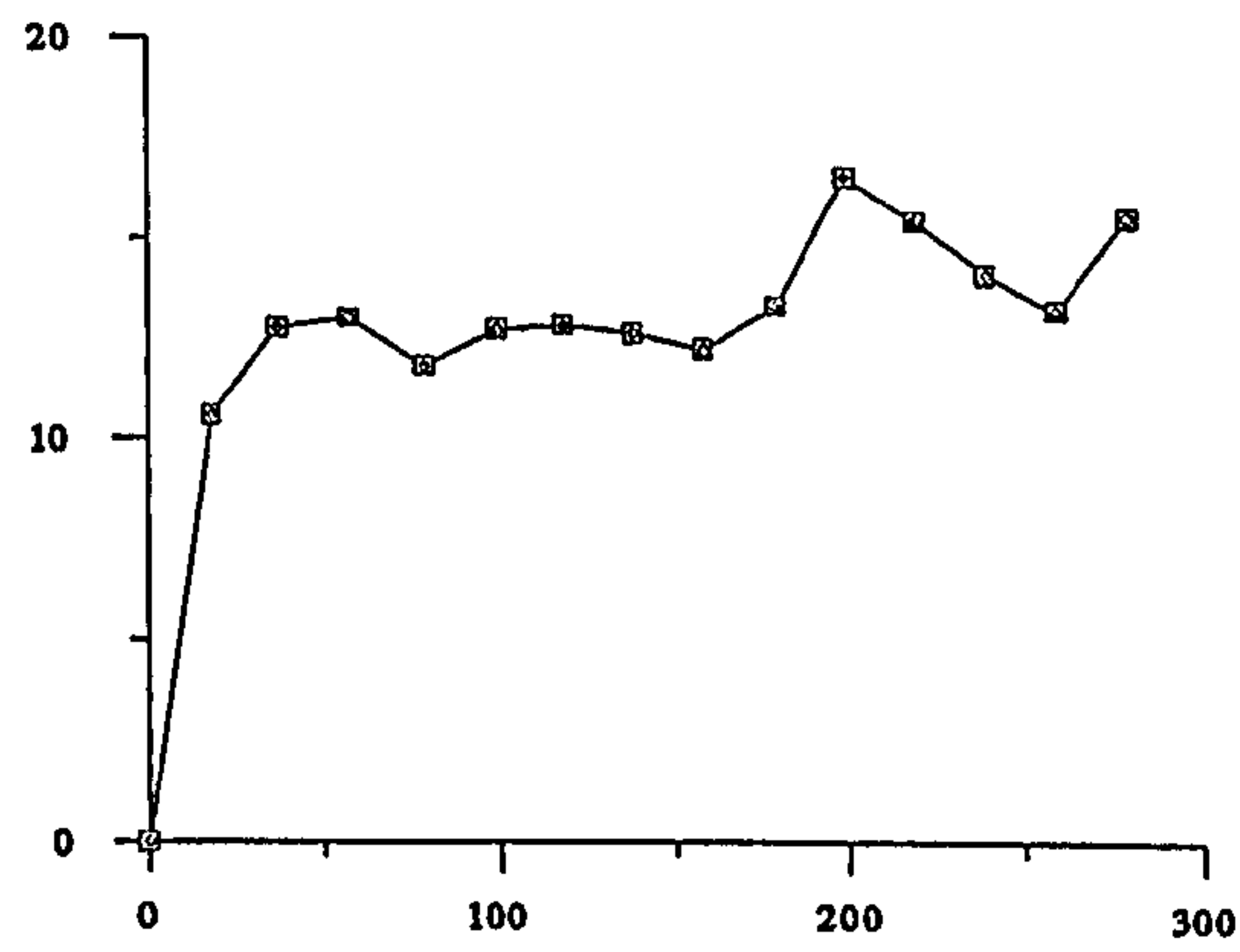


Fig 2.4 Variograms of soil Mg²⁺, Fe³⁺ and Ni²⁺ concentrations constructed from the 228 samples collected in May 1992.



pH



Stoniness

Fig 2.5 Variograms of soil pH and stoniness constructed from the data collected in May 1992.

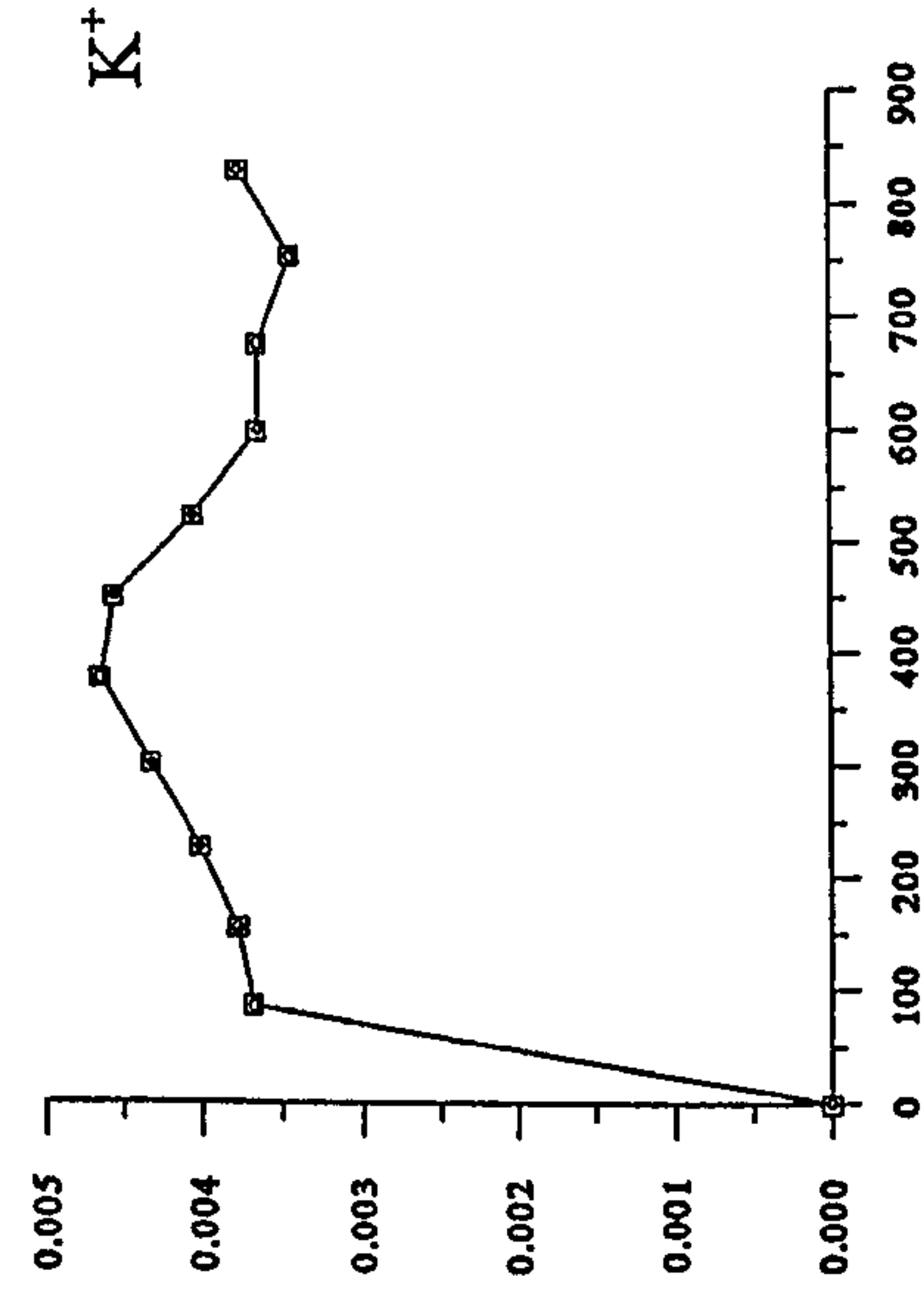
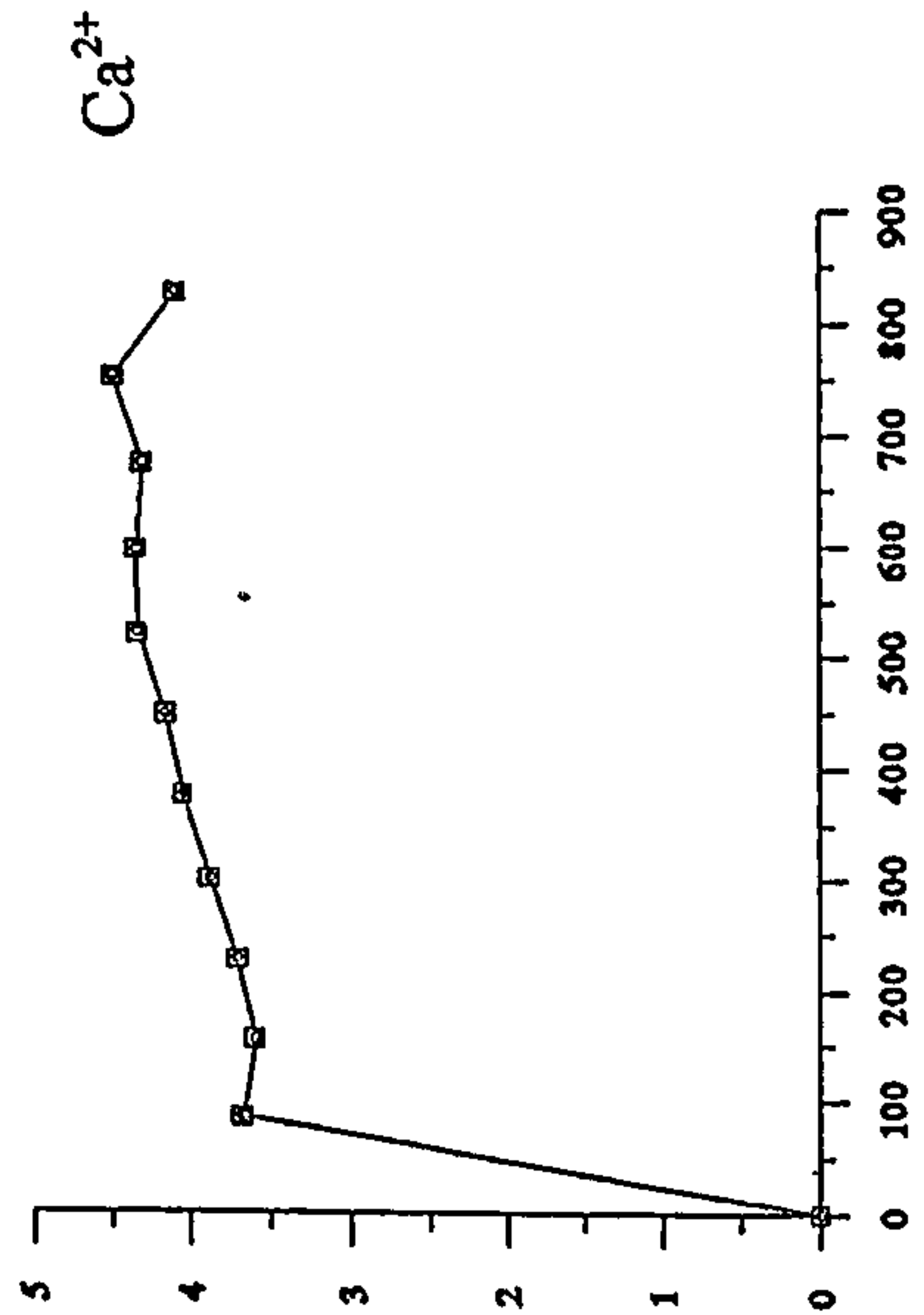
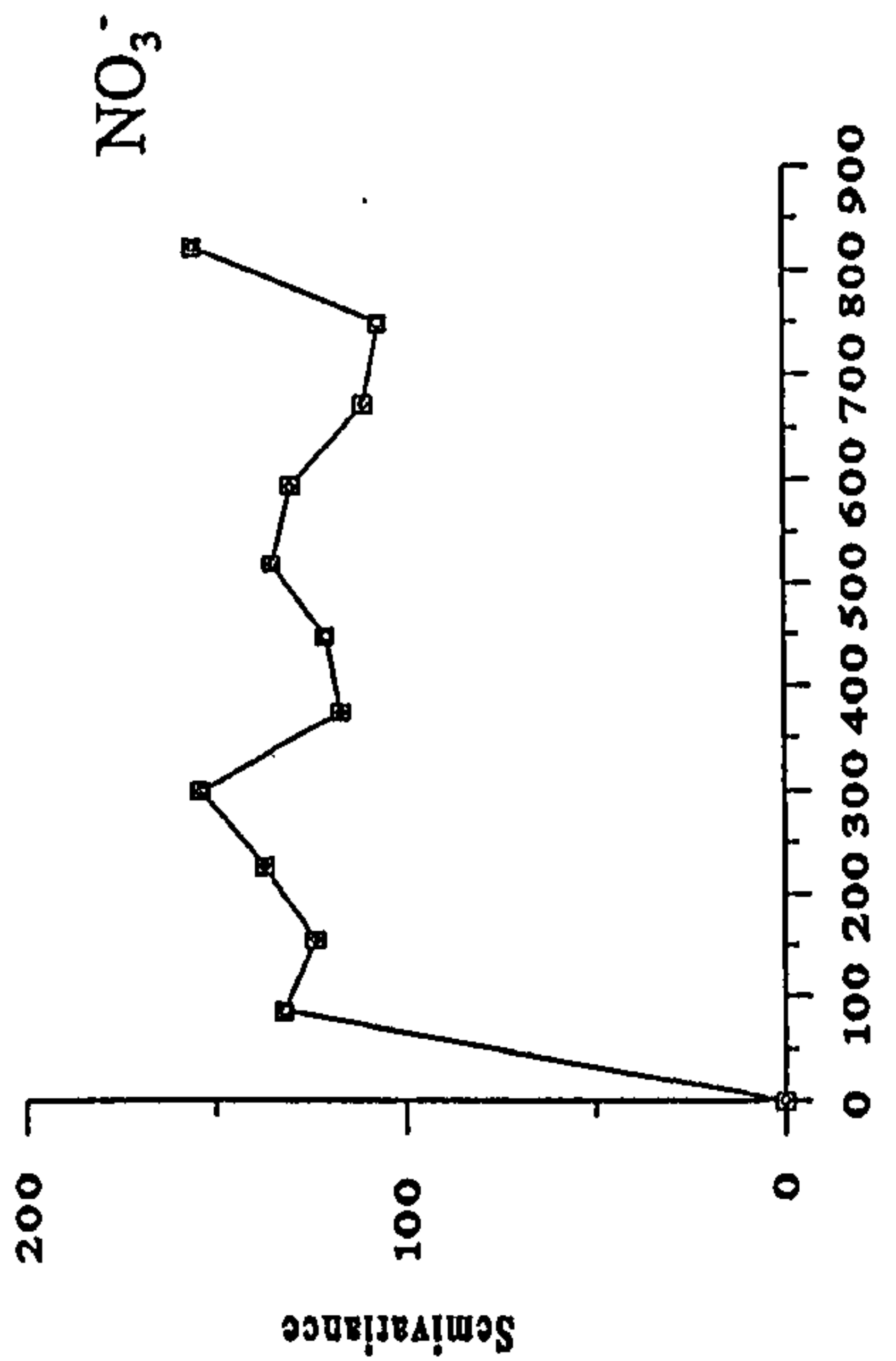
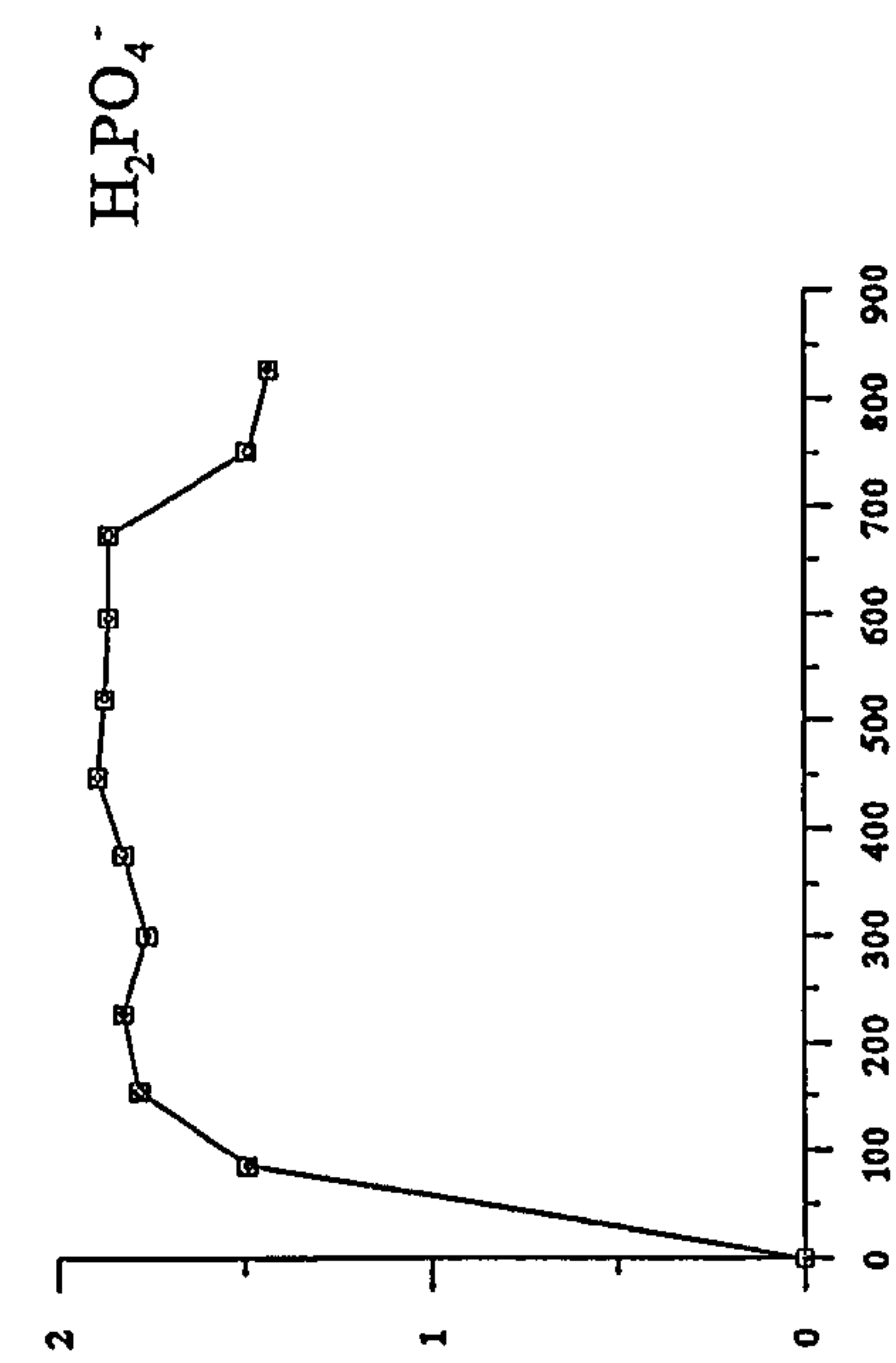


Fig 2.6 Variograms of the concentrations of NO_3^- , $H_2PO_4^-$, K^+ and Ca^{2+} constructed from the 144 samples collected in October 1992.

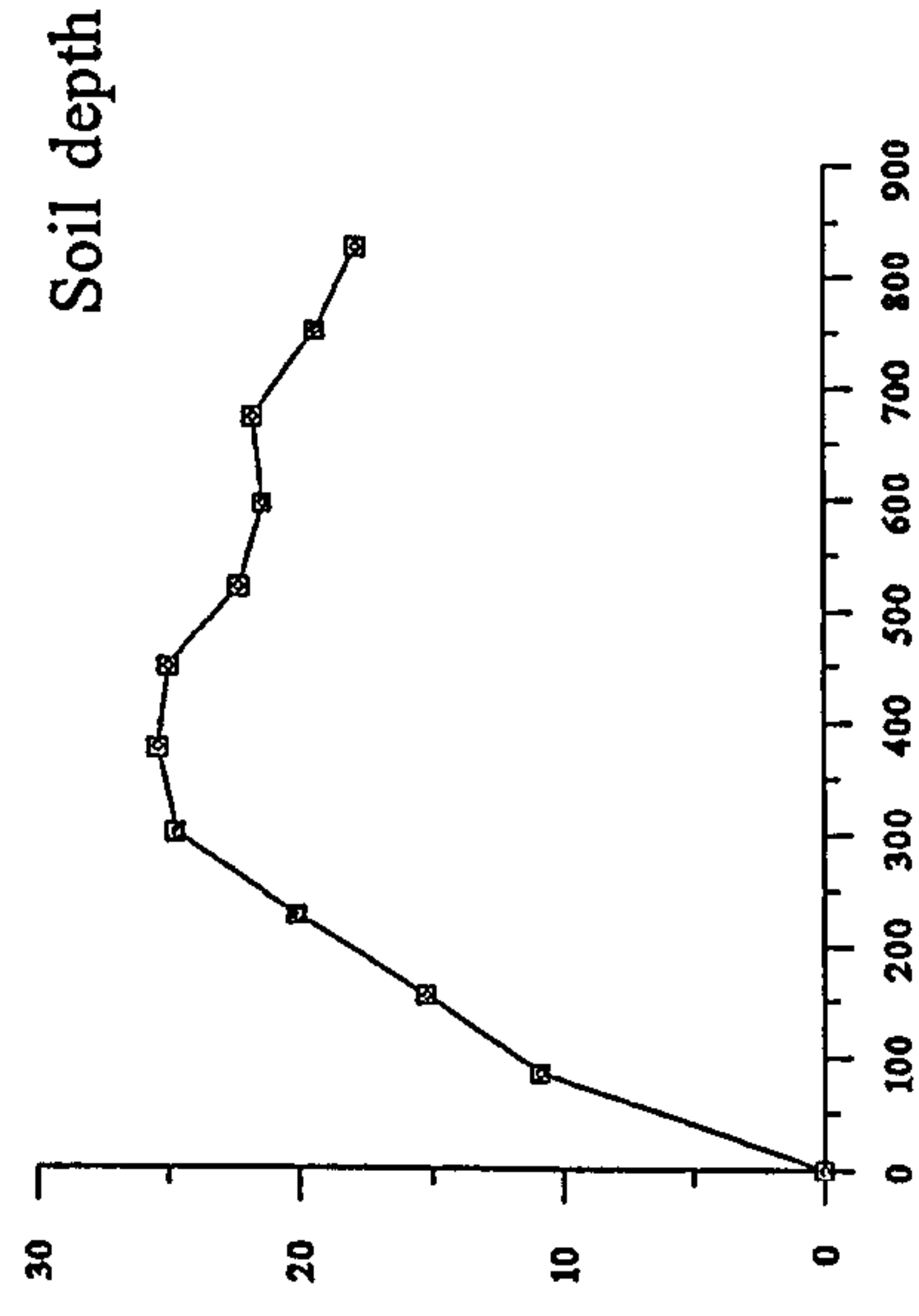
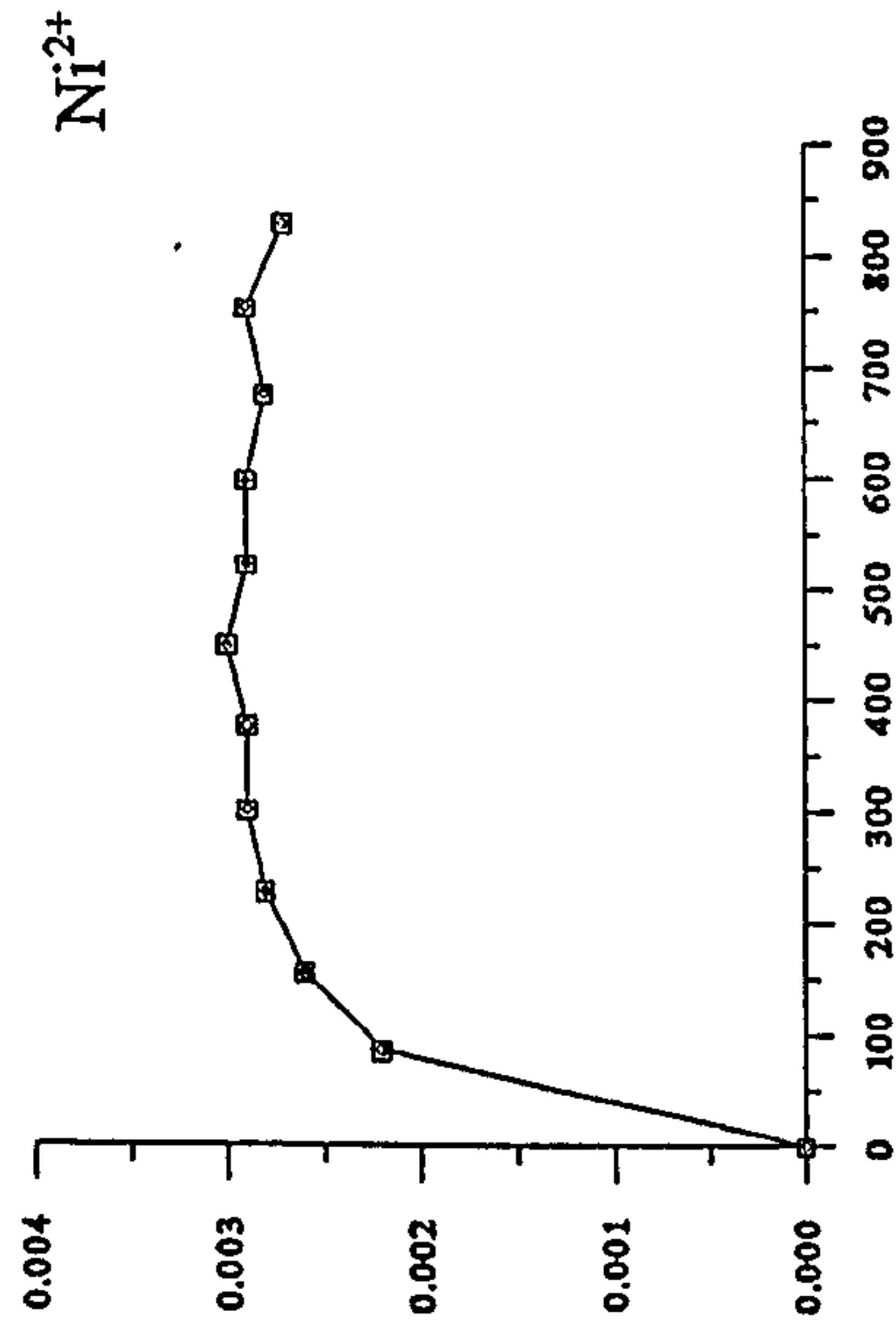
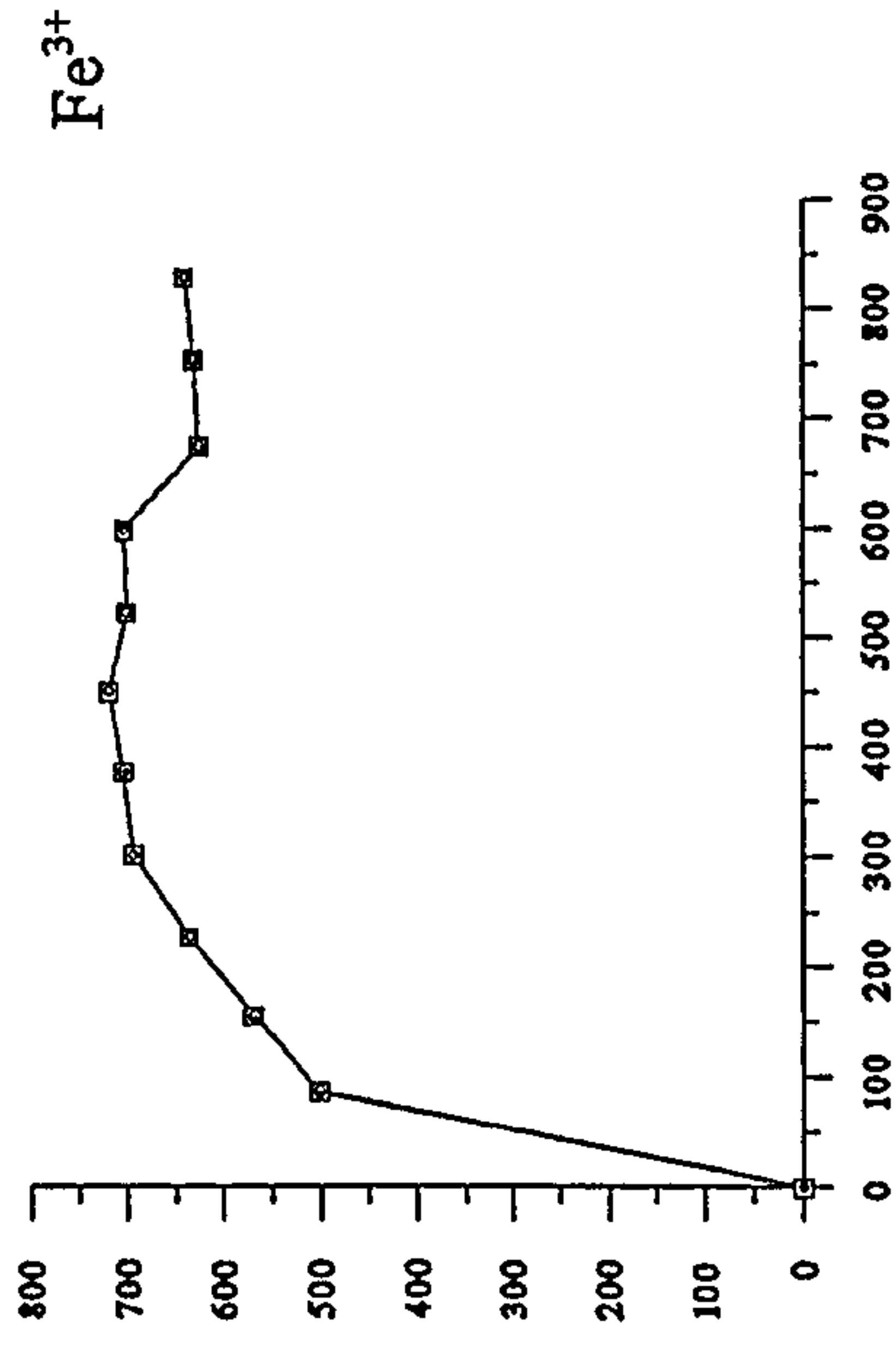
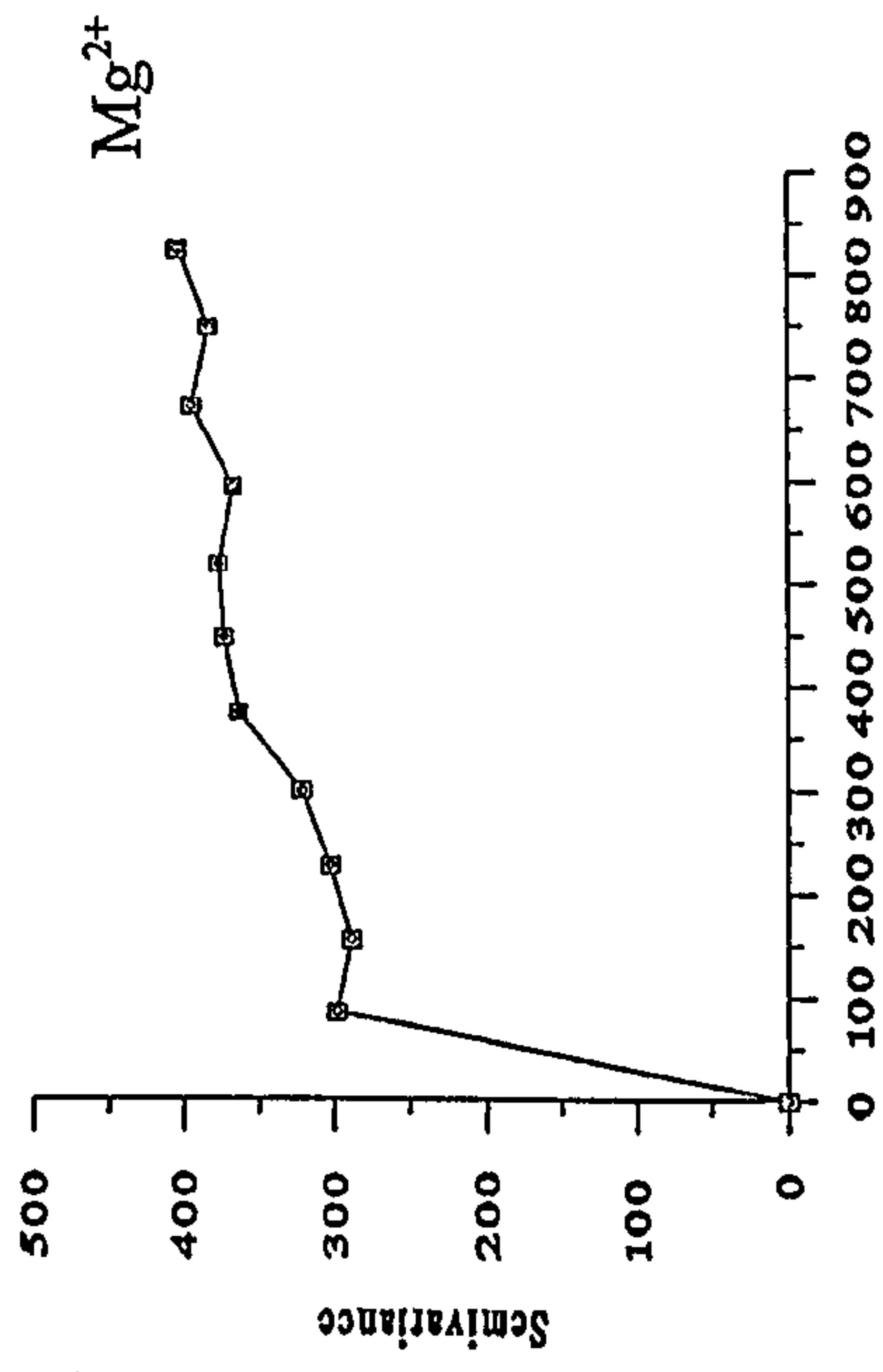
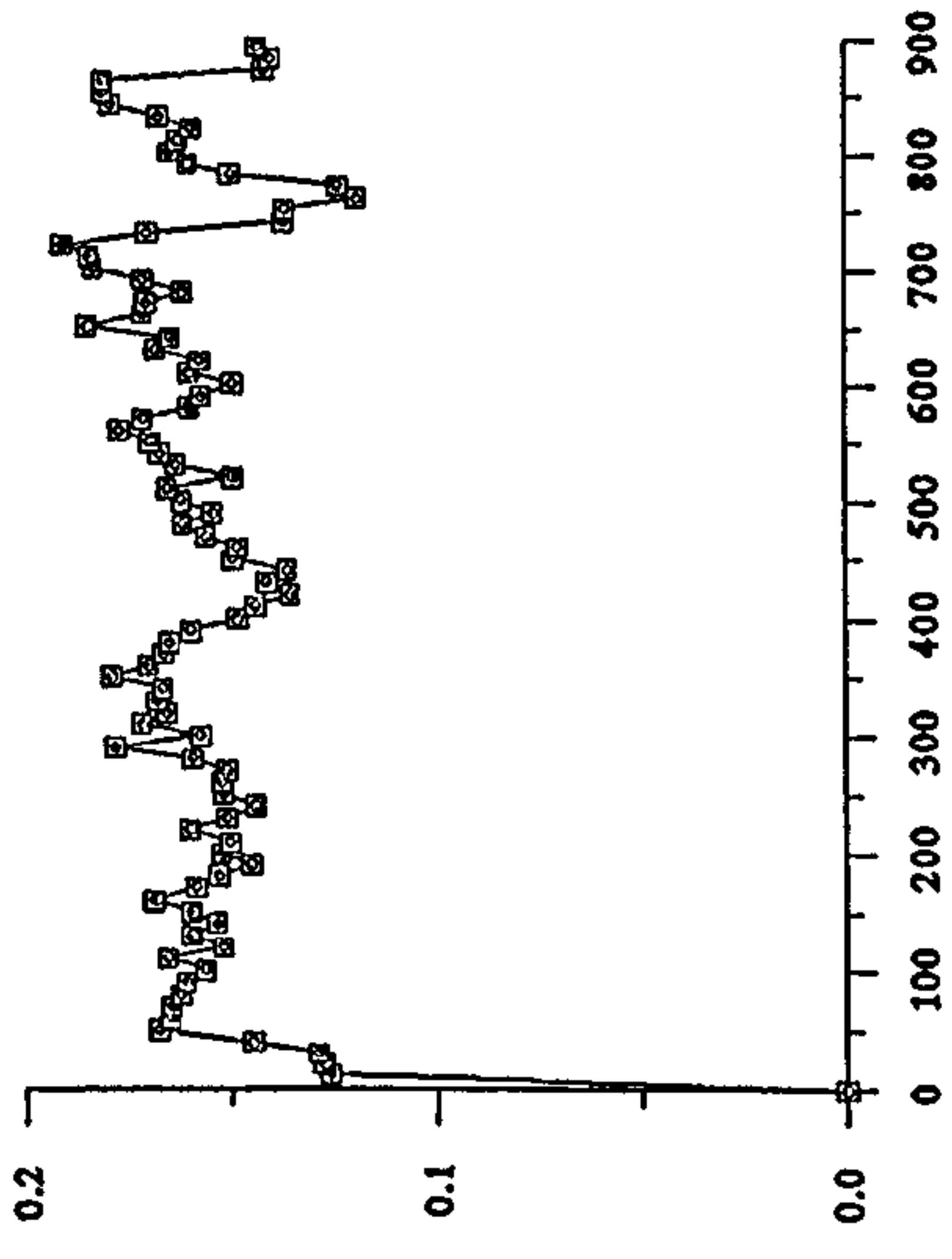
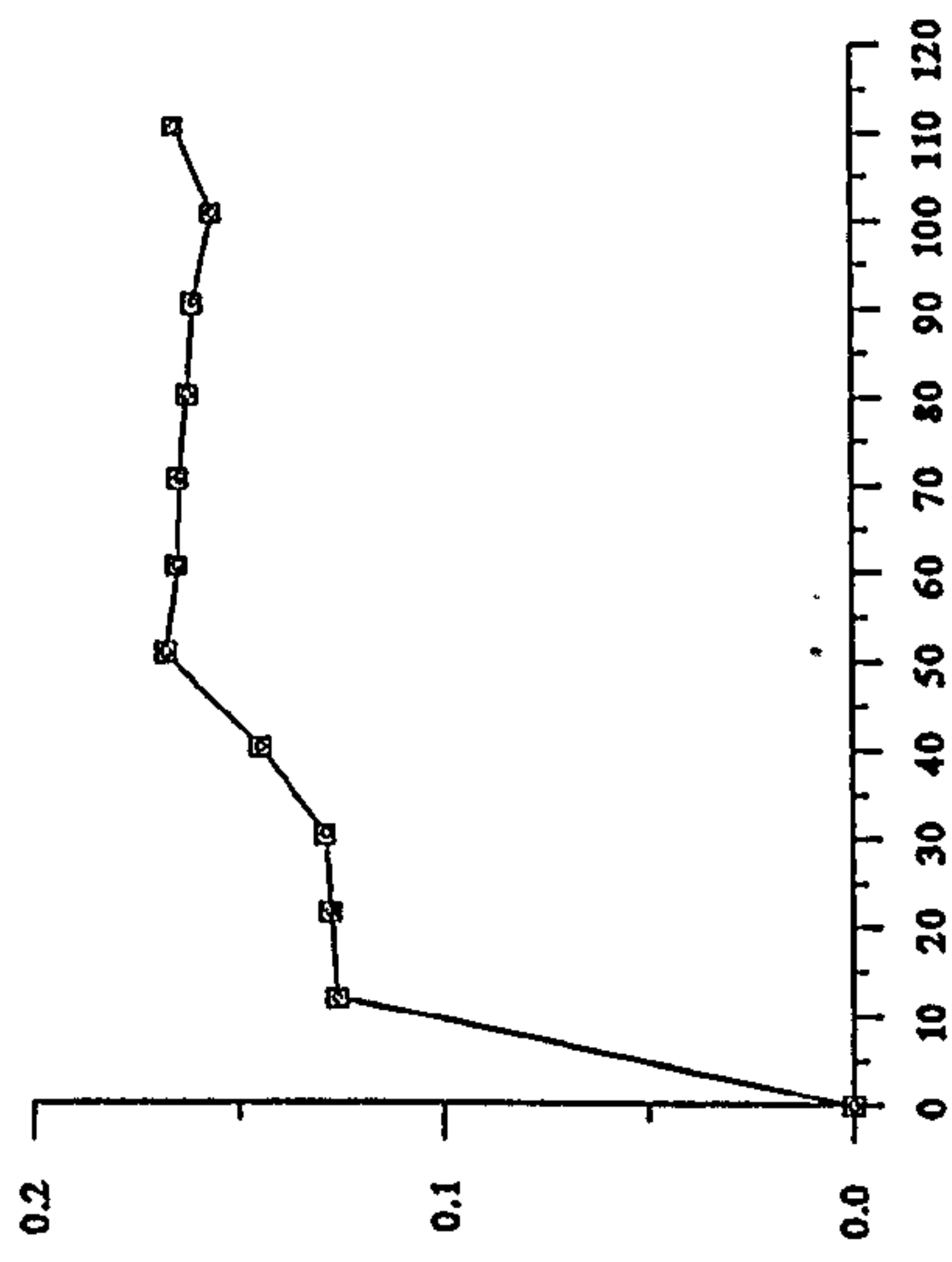


Fig 2.7 Variograms of the concentrations of Mg²⁺, Fe³⁺, Ni²⁺ and soil depth constructed from the 144 samples collected in October 1992.

Armeria maritima



Agrostis vinealis

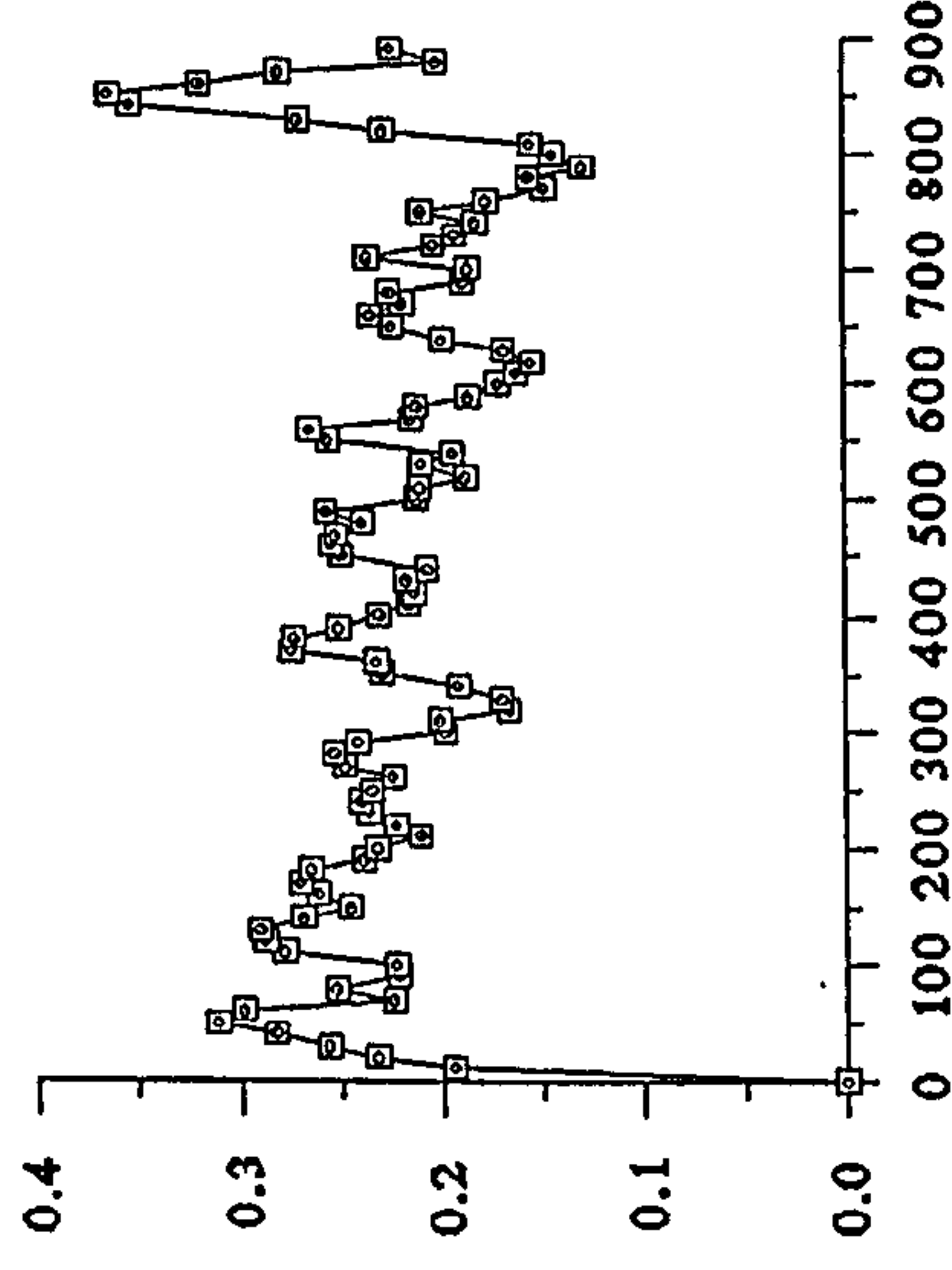
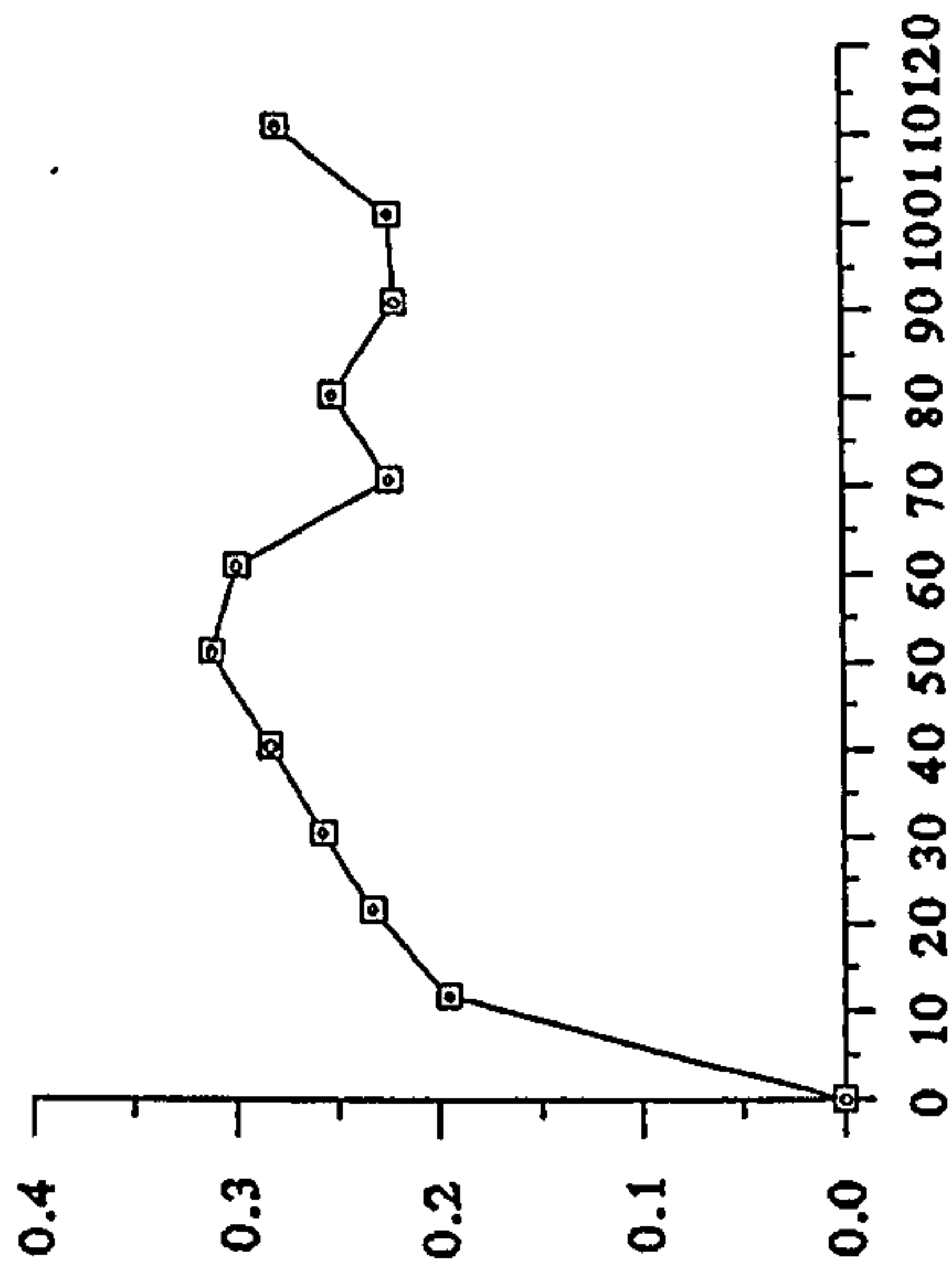
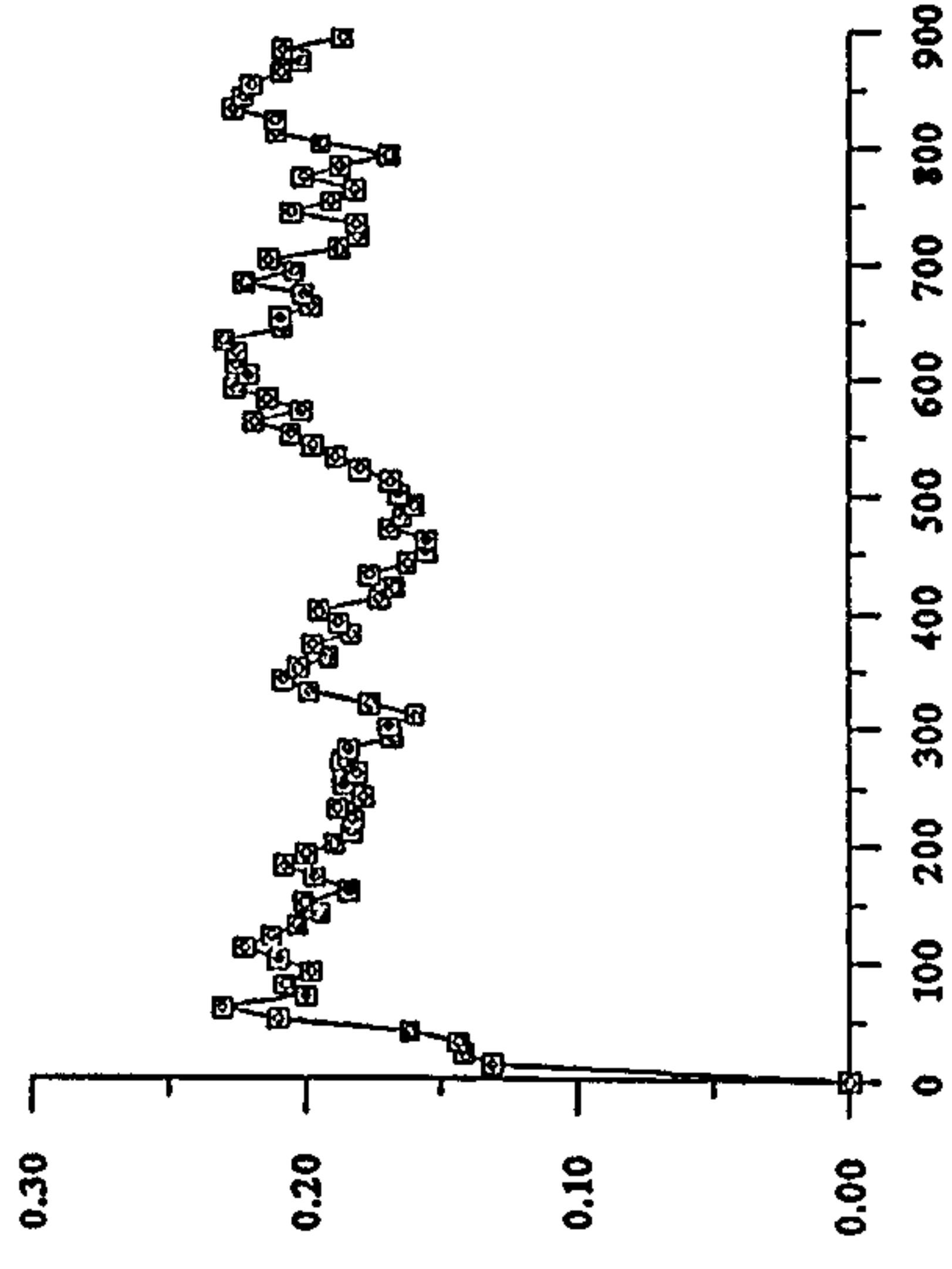
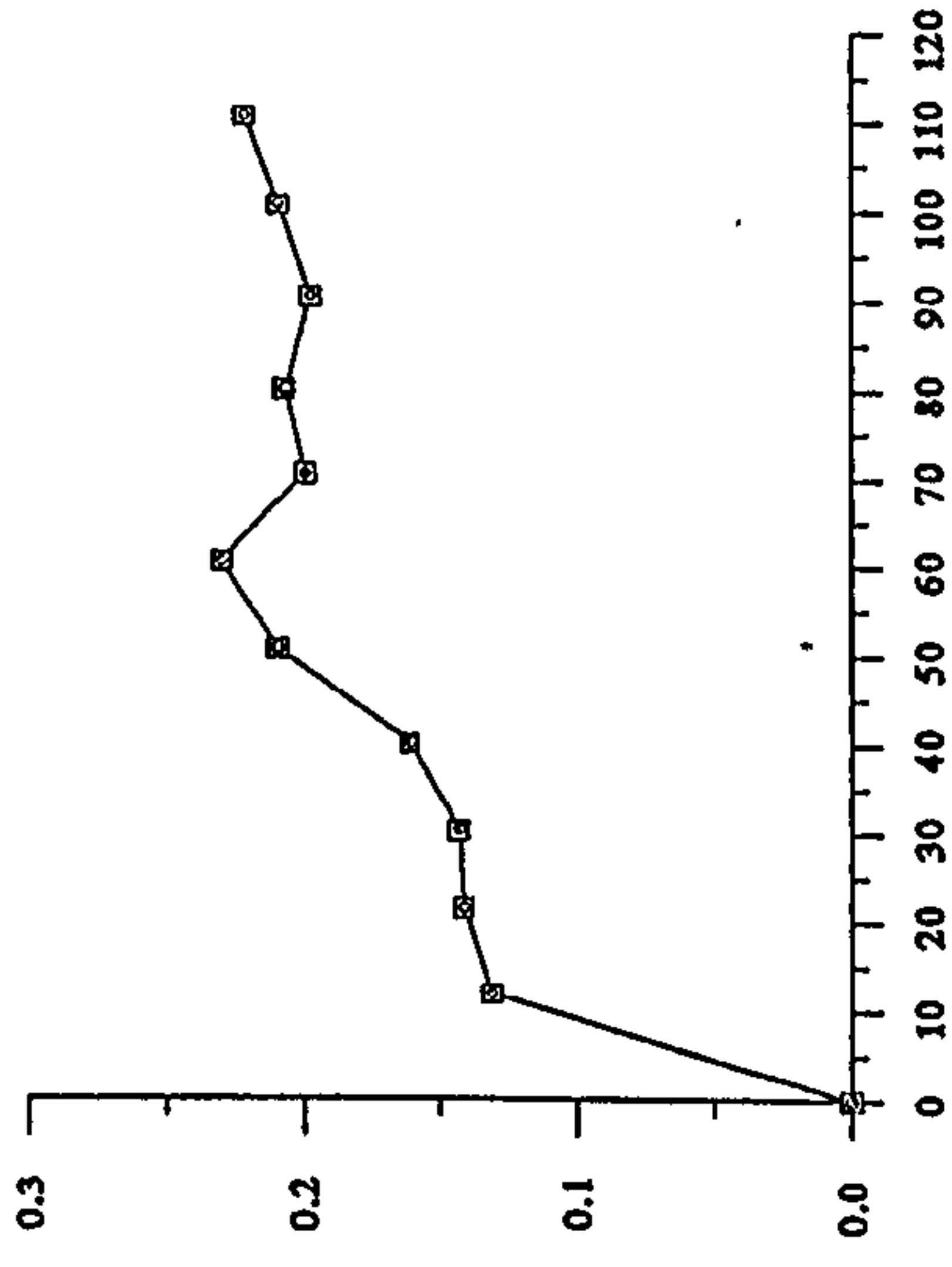


Fig 2.8 Variograms constructed for Armeria maritima and Agrostis vinealis at the 0-300 and 0-900 cm scales at MKI in 1992.

Cerastium fontanum ssp. scoticum



Cochlearia pyrenaica ssp. alpina

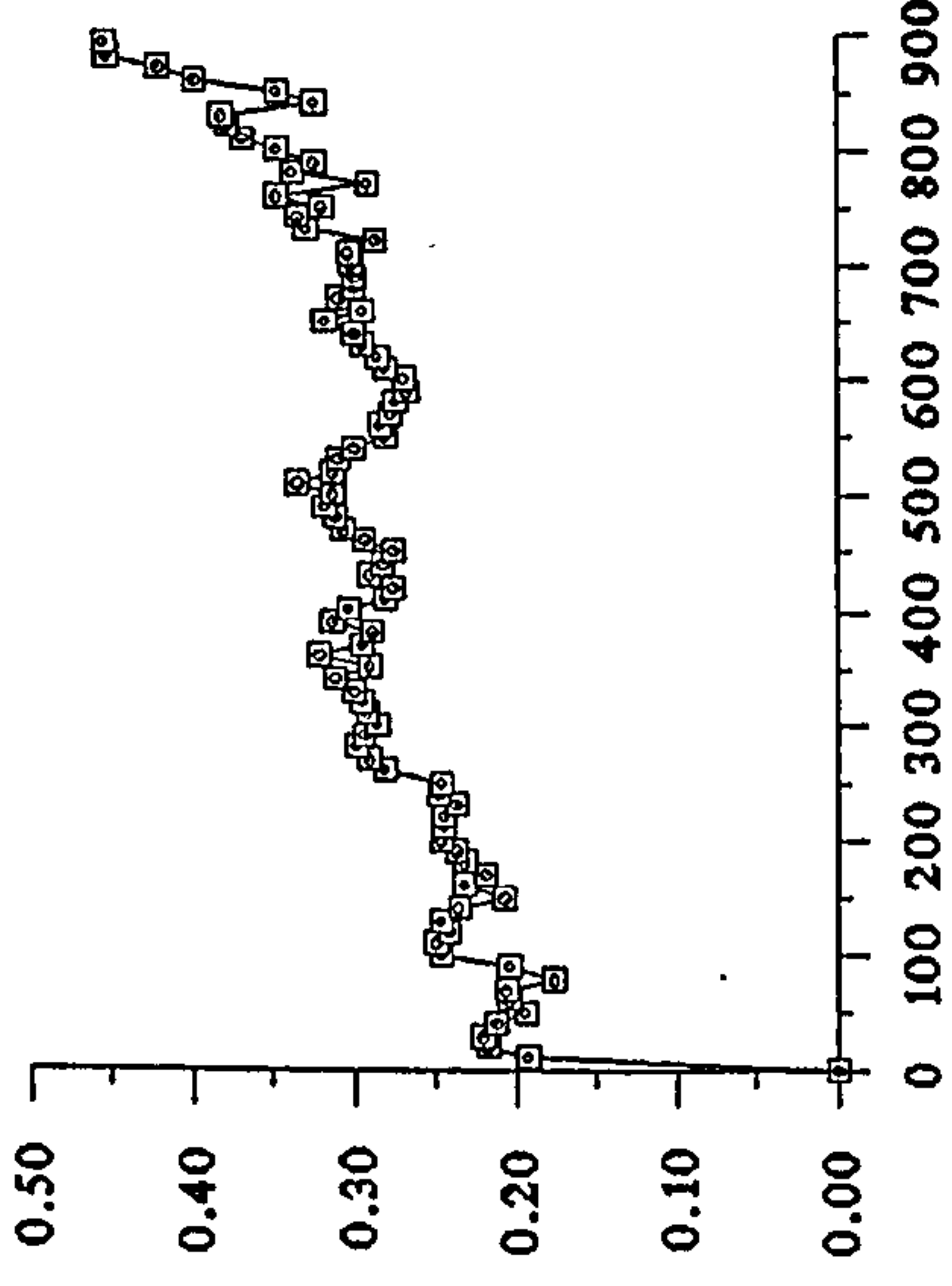
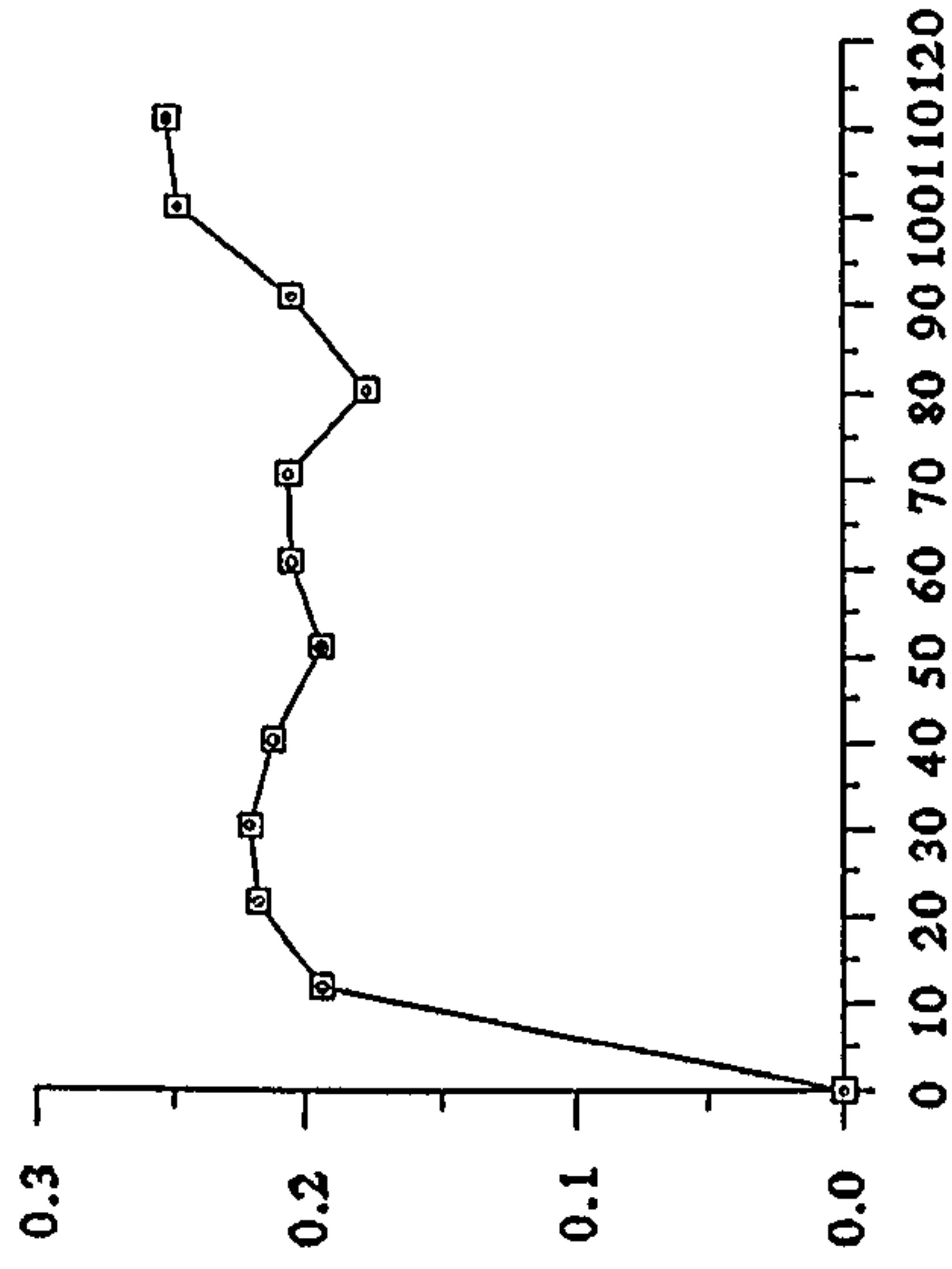
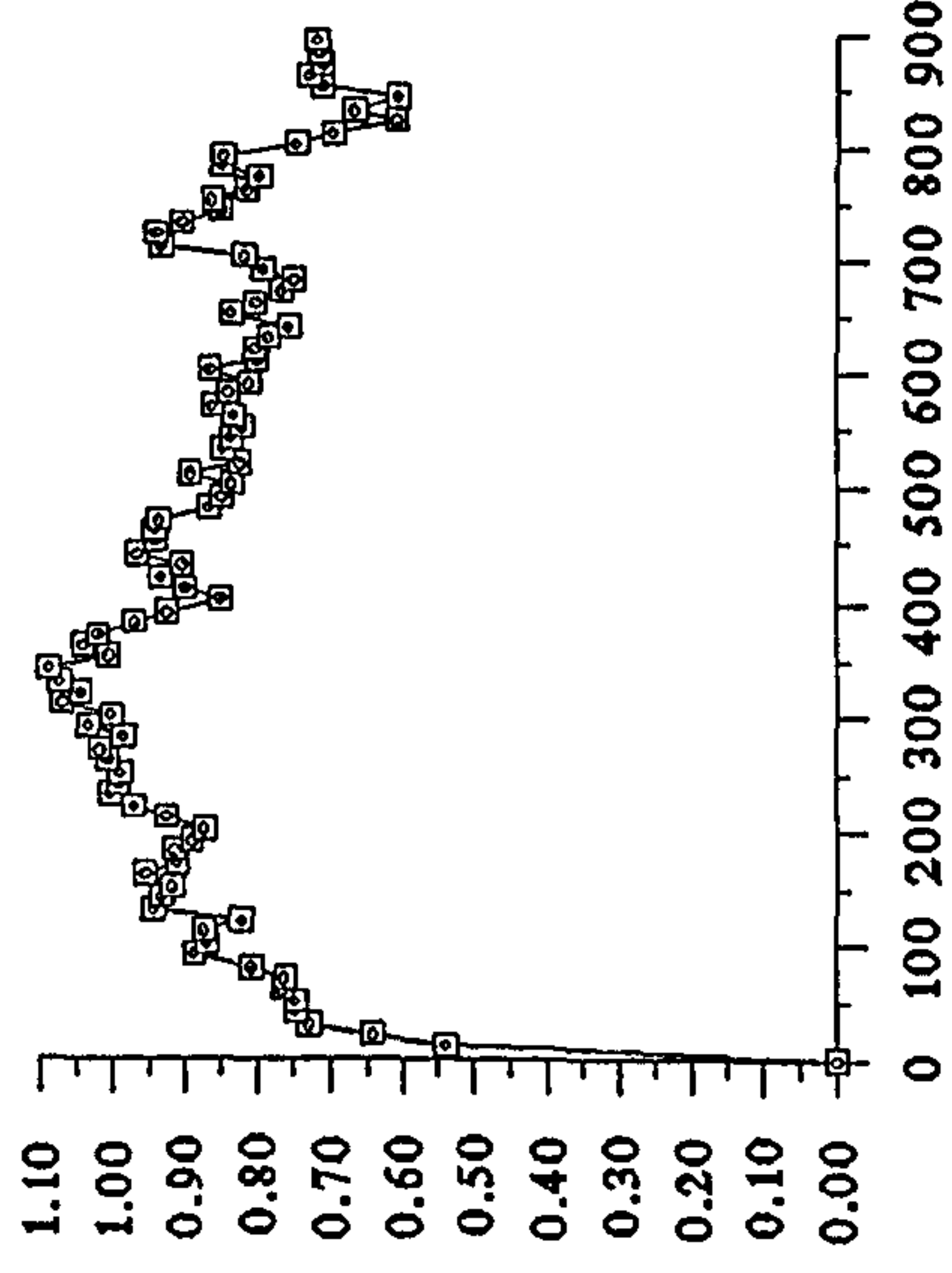
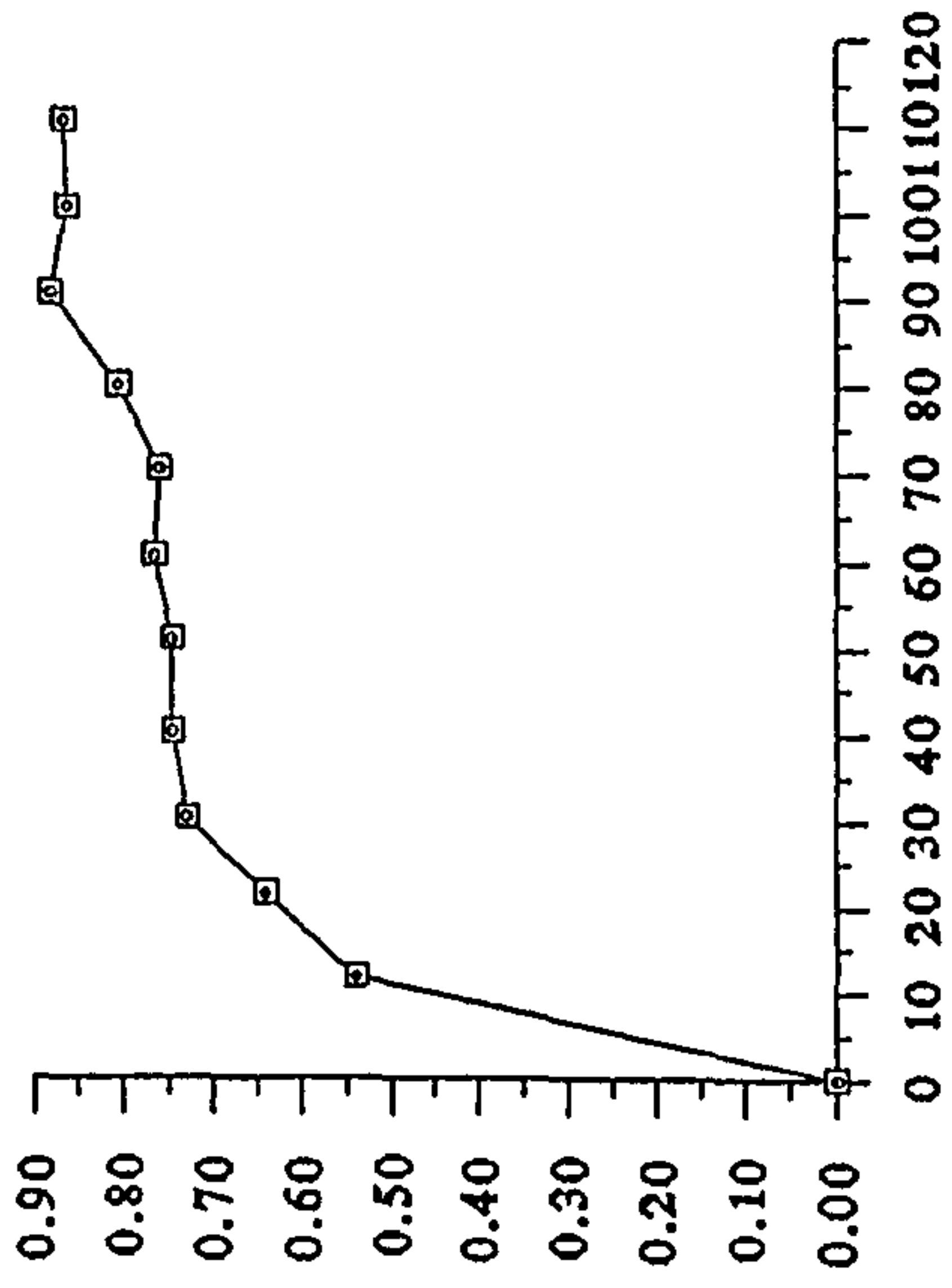


Fig 2.9 Variograms constructed for Cerastium fontanum ssp. scoticum and Cochlearia pyrenaica ssp. alpina at the 0-300 and 0-900 cm scales at MK1 in 1992.

Festuca rubra



Lychnis alpina

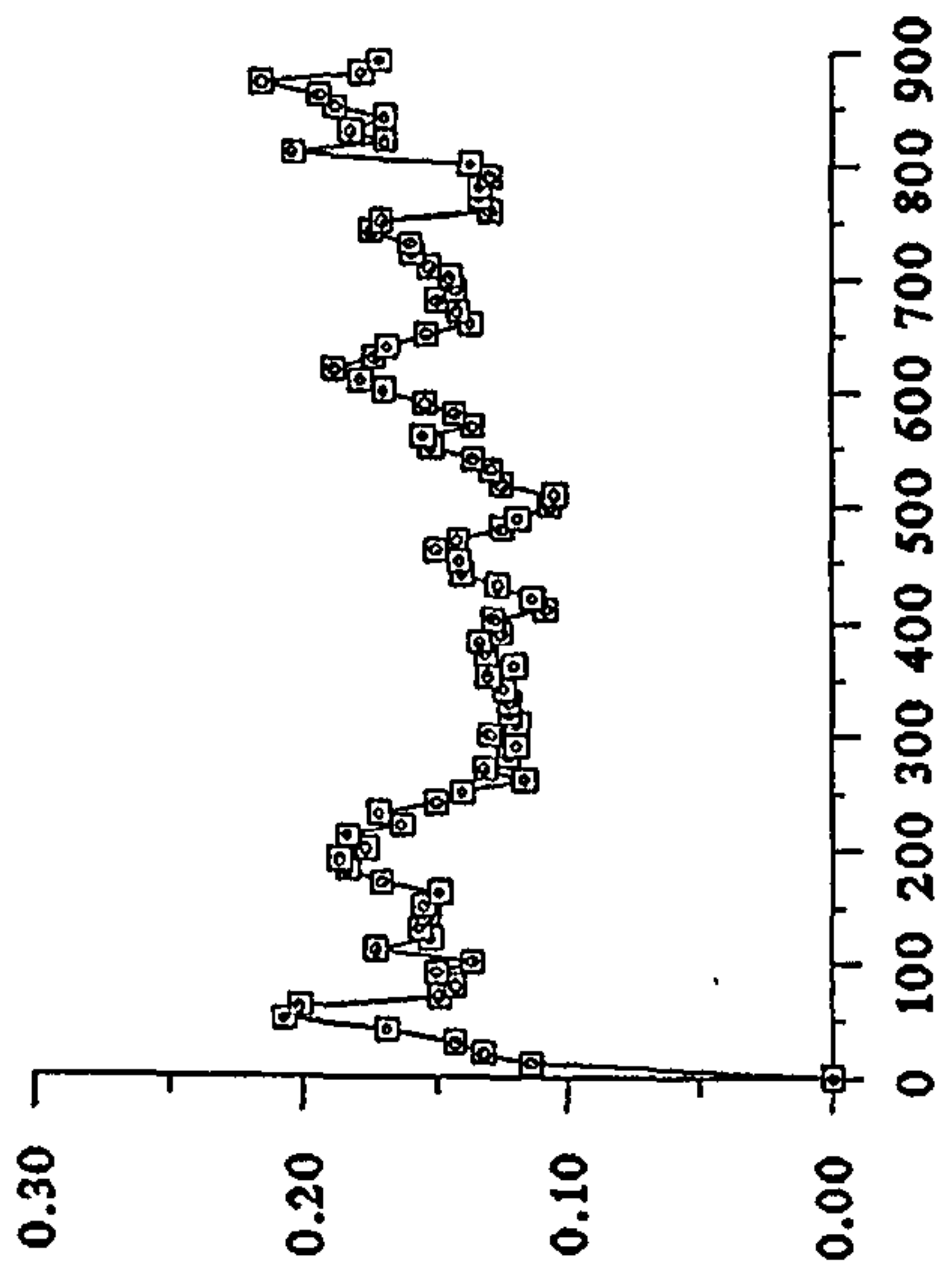
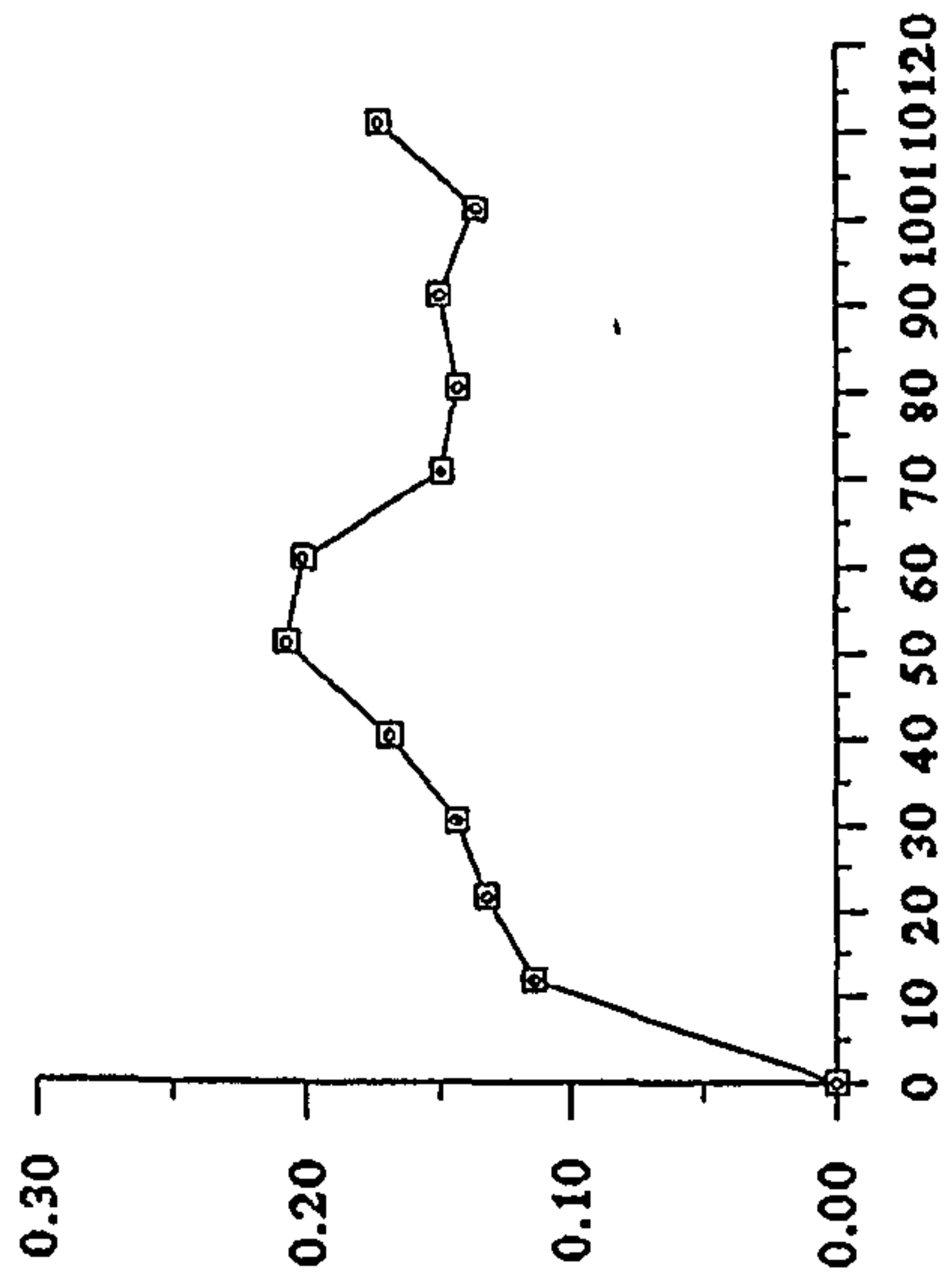


Fig 2.10 Variograms constructed for Festuca rubra and Lychnis alpina at the 0-300 and 0-900 cm scales at MK1 in 1992.

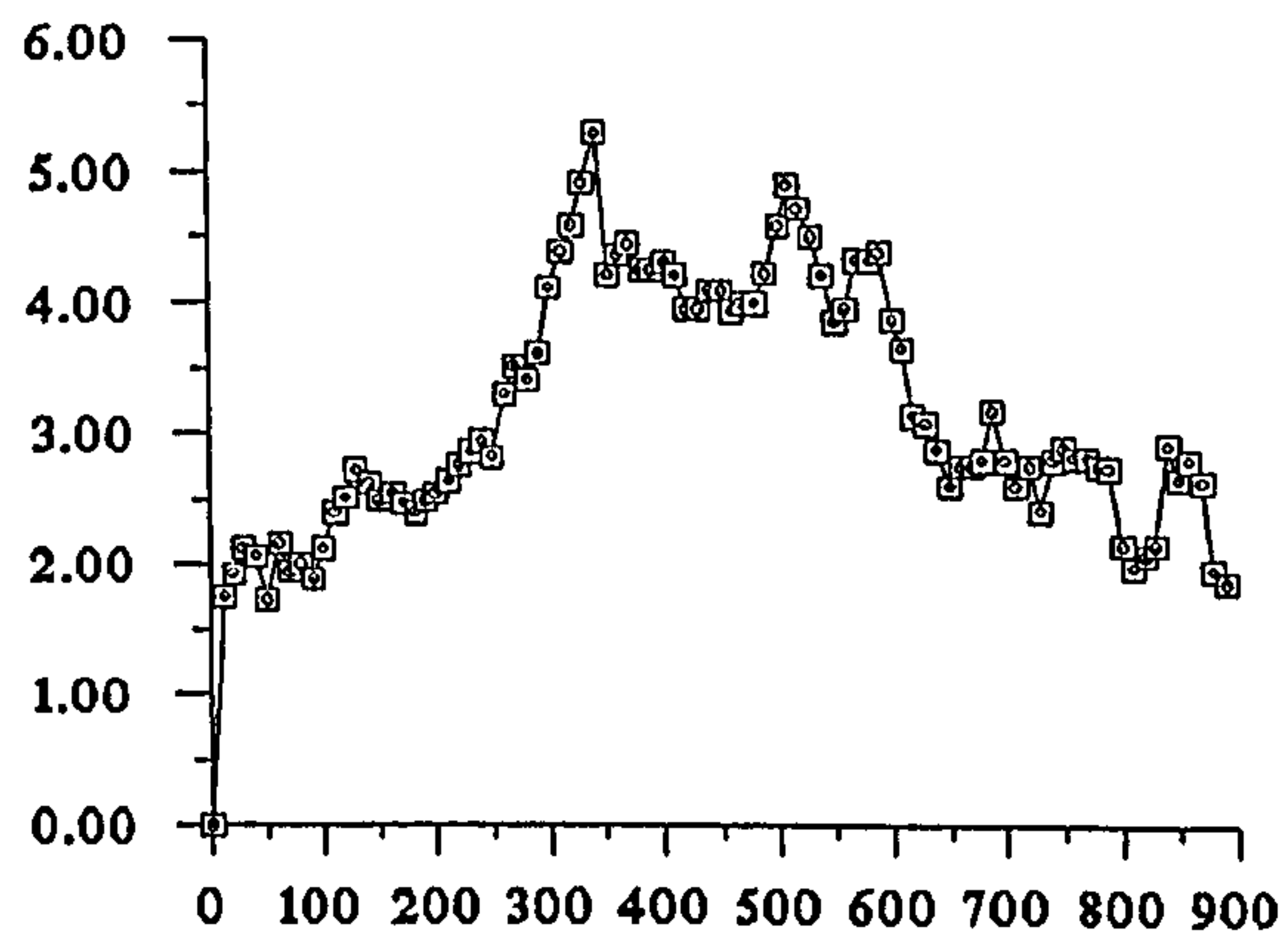
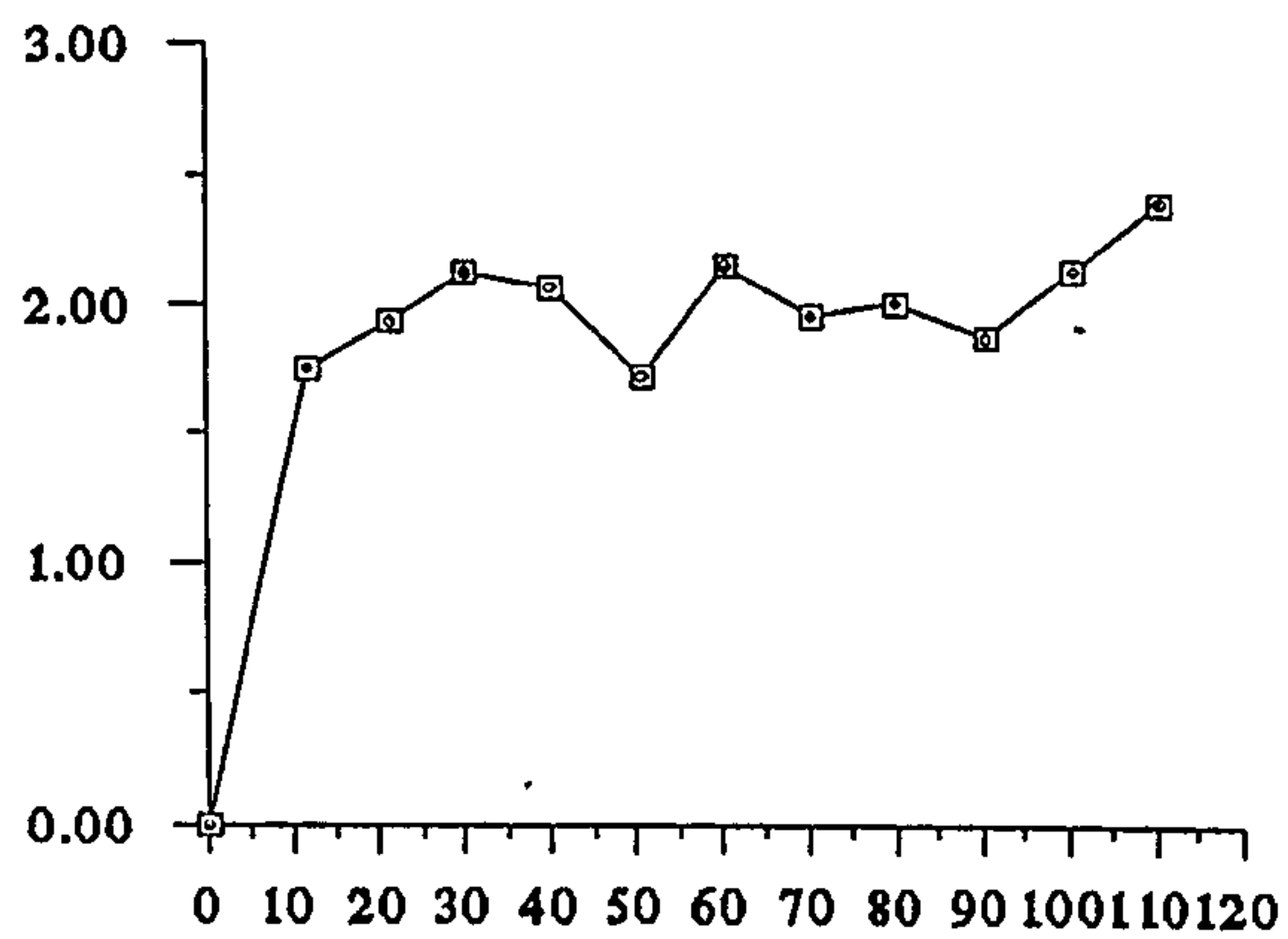


Fig 2.11 Variograms constructed for total vegetation data using values of axis 1 of a non-centred, non-standardised PCA on log-transformed data.

	H ₂ PO ₄ ⁻ (mg l ⁻¹)	K ⁺ (meq)	Ca ²⁺ (meq)	Mg ²⁺ (meq)	Iron (mg l ⁻¹)	Ni ²⁺ (meq)	Stoniness (Relative units)
Q1	0.11a	0.07a	0.67a	15.0a	8.7a	0.06a	16.6a
Q3	0.10a	0.05a	0.65a	13.9a	18.0ab	0.10a	14.8ab
Q7	0.10a	0.12b	1.29b	27.8b	17.6ab	0.09a	12.6b
Q10	0.12a	0.10ab	1.31b	25.7b	34.3bc	0.17b	16.5a
Q18	0.13a	0.14b	1.21b	29.6b	27.6c	0.17b	15.2ab
Q26	0.17a	0.11b	1.62bc	30.4b	35.0c	0.21b	12.7b
Q34	0.10a	0.11b	1.85cd	40.9c	35.0c	0.27c	12.4b
Q36	0.27b	0.10ab	1.89d	37.9c	76.6d	0.31c	14.4ab

Table 2.5 Mean concentrations of ions and mean values for stoniness in the eight sub-plots determined in May 1992 (n=25). For each column: different letters indicate significant differences ($p \leq 0.05$); values with identical letters are not different.

up to 4 m (Fig. 2.7). A variogram for Ni^{2+} was only constructed at the small scale because of an inexplicable decreasing trend with increasing separation distance in semivariance values at the larger scale (Fig. 2.4). Soil depth showed a clear increase in semivariance to 390 cm (Fig. 2.7), while stoniness appeared to follow no degree of continuity beyond 40 cm (Fig. 2.5). pH showed spatial dependence up to 150 cm (Fig. 2.5).

The variograms for the plant species showed different extents of spatial dependence (Figs. 8-10). At the smaller scale, Agrostis exhibited a high level of spatial dependence up to 40 cm, indicating patchy growth of that size (Fig. 8). When examined at the larger scale, however it appeared that overall there was no spatial dependence and the distribution of the species was rather random with peaks and troughs of spatial periodicity alternating at distances of about 50 cm. (An aggregated structure recurring at intervals will show spatial dependence for distances corresponding to the gap between patch centres (Legendre & Fortin 1989). For Festuca after the 40 cm separation distance, there was no further increase in the variance up to about 1 m. There was a peak at 3 m and another at 7 m, with decreasing variance values for the distances in between, indicating that Festuca probably grows in patches of 3-3.5 m (Fig. 10). At the small-scale, Armeria (Fig. 8), Cerastium (Fig. 9) and Lychnis (Fig. 10) showed spatial dependence up to 70 cm, 60 cm and 40 cm respectively, although at larger scales they were nearly random. Cochlearia did not show any degree of pattern at the small scale, while at the larger scale there was an indication of spatial dependence at a scale larger than the total extent of the study area (Fig. 9).

Variograms for the total vegetation showed that three scales of pattern were discernible: 0-30 cm; 0-150 cm and 0-320 cm patch sizes (Fig. 11). There does not appear to be any

indication of the existence of larger vegetation units beyond 320 cm.

Discussion

Values for resin-extractable K^+ , Ca^{2+} and Mg^{2+} were similar to those obtained by Johnston & Proctor (1981) in their soil solution extracts. There were large differences in NO_3^- and $H_2PO_4^-$ (ten to twenty-fold lower values for resins) and in iron and Ni^{2+} (one hundred-fold and five-fold higher values for resins). To be able to assess ion availability measured by ion-exchange resin extracts in comparison with traditional methods, the hydrological balance at the sample area for the period of sample collection would be needed. Also, a calibration experiment using bioassay plants would be required. This is however not the concern of the present study because values for each sample point were obtained by using the same method and therefore results are comparable.

The principal aim of the study was to characterise soil spatial variation at two different scales, one relevant to growth form, seed dispersal and establishment (10 cm to about 50 cm) and one for vegetation patchiness (>50 cm). It could be demonstrated that although there was a high point-to point variation at the 10 cm scale in the 50 cm x 50 cm sub-plots at the May sampling, the values for all cations increased significantly between sub-plots down-slope (Table 2.5). The range of spatial dependence is a function of the scale used in a study. Ranges for soil properties quoted by Trangmar *et al.* (Table 1, 1985) were between 4-6 m where the sampling distance was similar to that used in the present study.

The soil environment for many chemical factors was similar over distances of 0-3 m.

This is in agreement with reports by other workers (Lechowicz & Bell 1991b, Palmer 1990, Kelly & Canham 1992). The nugget variance was high for most of the soil variables (Table 3). This could have been caused by the inherent random variation at the micro-scale, probably as a result of cryoturbation. The sample collection effectively averaged the 7 cm x 7 cm area of contact of each resin bag. This variation in soil properties at the micro-scale is unlikely to have any effect on plant growth phases other than establishment. Then the very small scale variation may have an influence.

Strong spatial dependence of 30-60 cm was found for plants by examining the variograms. It is noticeable that the nugget variances were all about 50% (Table 4). This unaccounted variance may be due to error arising from variation at a smaller scale. It is probable that the level of spatial dependence is the highest at scales smaller than 10 cm, in other words, the high variance values at h_1 may reflect true clumping of plants in at least some of the species.

In answering the questions 'How far can habitat differences account for less intense and small-scale pattern?' and 'What other mechanisms may cause or reinforce spatial heterogeneity or patchiness?' Greig-Smith (1979) enlisted and evaluated the possible roles of a number of abiotic and biotic factors. The relevance of these factors for the present study requires review:

- Animals: there is certainly a role for animals (deer, mountain hare and ptarmigan) in shaping the patchiness of the site on the small (0-30 cm) and small-medium scales (50-300 cm) at least transiently. There is evidence for local high nutrient input by these animals which, considering the nutrient-poor status of the soil, could have profound effects on soil nutrient dynamics and plant growth. Also they should be reckoned with as potential herbivores and, in the

case of deer, potential sources of disturbance.

Interrelationship between plants: although plant density is low, interactions cannot be ruled out altogether (see Chapter VI). The way these interactions may contribute to pattern however is difficult to envisage.

Physical disturbance: cryoturbation and stone sorting and erosion occur regularly and enhance chance events. In areas with larger stones, it is likely that plants of relatively higher stature (e.g. Cerastium and Festuca) will be more frequent because the leeward side of the stones can provide shelter from high winds.

It has already been pointed out that some of the species have inefficient seed dispersal, while for the grasses, the growth form resulted in the small-scale pattern.

Many studies demonstrated that vegetation itself affects the soil environment (e.g. Gibson 1988, Hook et al. 1991, Jackson & Caldwell 1993) and therefore perceived patterns are determined by both inherent soil characteristics and by vegetation-soil interactions in an age-dependent fashion. The scales at which spatial dependence was apparent were similar for both some of the soil chemical characters (e.g. K^+ and iron) and vegetation (Armeria, Cerastium, Lychnis). It can be hypothesised that those edaphic factors which show similar level of spatial dependence to that of the vegetation may be correlated and may be causal, as for example, Robertson et al. (1988) suggested for the spatial heterogeneity of nitrogen availability and community structure in an old field. The root systems of the plants at MK1 are of similar dimensions to those reported by Spence (1957) for the Keen of Hamar, Unst, Shetland. The conditions that individual plants growing together within the observed range of spatial dependence are likely to experience are probably more definable than the 'average' conditions individuals were found to encounter in the study by Jackson & Caldwell (1993).

Those chemical factors of which the scale of spatial dependence exceeds that of species/vegetation patterns can be proposed as non-effective at the given scale. It also should be emphasized that while one factor may coincide in extent with that of the extent of patterns in vegetation, others can be randomly occurring (Kelly & Canham 1992). In many cases therefore the cross-product of those factors may be of higher local importance for plant establishment and early growth while later growth, spread, and distribution are more likely to be determined by structural variation (pattern) in the soil (Palmer 1990, Palmer & Dixon 1990). The consequences of the non-random variation (patchiness) in the soil environment have also been clearly demonstrated for maintaining intrapopulation variation (Lechowicz & Bell 1991, Lechowicz *et al.* 1991, Bell & Lechowicz 1991).

Spatial dependence (pattern) could be shown in the present study for both some soil features and for most of the plant species. The following chapter (Chapter III) will further explore the soil - vegetation relationship at MK1 by using ordination techniques.

Chapter III

Soil micro-spatial variation and vegetation patterning on the fellfield type skeletal soil at MK1. II. Correlations between vegetation and the soil environment and their scale dependence

Introduction

This chapter deals with two main topics: correlations between the vegetation and soil environment; and the scale dependence of pattern and of the vegetation and environmental correlations.

The main interest for this study was the β -diversity (the change in species composition along environmental gradients), though the use of different grain sizes for scaling will require the discussion of the other types of pattern (Chapter II). (In this thesis 'grain size' is used interchangeably with 'quadrat size' and 'resolution'; 'scale' always refers to linear distances; and 'extent' is used for the size of the sampling area.) It is generally assumed that differences in vegetation along gradients at scales larger than 1.5 m are determined by differences in single or multiple (compound) environmental factors effective at the appropriate scale. At smaller scales morphological traits and plant-to-plant interactions are more important. Any correlation found between vegetation and environmental factors can suggest causality but the use of additional information and 'commonsense ecological interpretation' are needed to formulate hypotheses about cause

and effect. Work on cause and effect often stops short of experimental confirmation and is thus open to criticism.

The scale dependence of pattern, both spatial and temporal, has long been recognised (Greig-Smith 1952, 1979). Perceived patterns can differ considerably in studies with different extents of area and grain size (O'Neill et al. 1986, Wiens 1989, Reed et al. 1993). The scale dependence of observations necessarily limits the inferences that can be made and the applicability of the results. Using a fine resolution will reveal a higher spatial heterogeneity than the use of larger units which will average out small-scale differences. Pattern often will not be detected if too fine a resolution is used, as the latter may lead to equal or greater heterogeneity between points than exists between larger units of observation. In other words, pattern may not be obvious because environmental variation within plots, which themselves are composed of small-scale units of observation, can be of similar, or higher magnitude than between plots (Palmer 1990). Using different grain sizes and extents to investigate if correlations between the environment and vegetation are similar over a range of scales has received recent attention (e.g. Allen & Hoekstra 1991, Reed et al. 1993).

The main objective of the work reported in this chapter was to determine to what extent the measured variables may explain the observed vegetation pattern (environment correlated pattern) and to use the correlations found to form a testable hypothesis as to the causes of the distribution of the species. It was of related interest to investigate how different grain size (100 cm² to 2500 cm²) and extent (3 m x 3 m and 9 m x 7 m) affected these correlations: environmental and vegetation data at the 100 cm² grain size were compared at the two extents; and grain sizes of 100 cm² to 2500 cm² at the 9 m x 7 m extent.

It was hoped that the information generated would help the conservation management of the site.

Material and Methods

Data presented in Chapter II were used in the analyses carried out in this study.

ESTIMATION OF ENVIRONMENTAL VARIABLES

Environmental variables presented in Chapter II were used to study the environment - vegetation correlations. Environmental variables for the six 50 cm x 50 cm sub-plots sampled only for vegetation in October 1992 were estimated using the kriging method introduced in Chapter II. The values for the 20 cm x 20 cm, 30 cm x 30 cm, 40 cm x 40 cm and 50 cm x 50 cm grain sizes were derived from maps constructed by the above method.

VEGETATION PATTERN - GRADIENT ANALYSIS

Indirect and direct gradient analysis methods were carried out to study the correlations between environmental variables and vegetation. Principal components analysis (PCA) and redundancy analysis (RDA) (the canonical form of PCA) were used to ordinate the data because the relatively small spatial scale involved suggested a linear rather than a unimodal response of species to environmental variables. The main feature of RDA is that it selects the best linear combinations of environmental variables that give the

smallest total residual sums of squares for ordination axes. The results of RDA's were compared with those of PCA's to obtain an understanding of how much of the total theoretically possible variance was explained by the environmental variables included in the analyses. (It is possible to obtain high environment - vegetation correlations for the ordination axes, but this may be of no importance in cases where the total variance explained is negligible.)

Two sets of data, representing the two different extents of area, one of 228 samples for May 1992 and one of 144 samples for October 1992, were subjected a species-centred PCA with standardisation by species norm. The transformations were used to increase the information value of the first axis (centring versus non-centring) and standardisation to allow for the different growth forms (rosette v. grasses) in the density data used (Greig-Smith 1983). Data were log-transformed to standardise variances. To allow for the possible 'block effect' (arising from the spatial autocorrelation in the vegetation and environment data) at the May sampling (eight 'blocks', each of twenty-five quadrats), covariables were included. The ordination of the vegetation data was followed by a regression of the environmental variables using CANOCO, ter Braak 1990). Finally an RDA was carried out using data transformations identical with those used in the PCA's.

Pattern and its correlation with environmental variables were studied at five grain sizes (10 cm x 10 cm, 20 cm x 20 cm, 30 cm x 30 cm, 40 cm x 40 cm and 50 cm x 50 cm) to investigate if the same environmental variables were correlated with vegetation at all resolutions. PCA's were carried out on data sets of fourteen vegetation samples for each of the five grain sizes. This was followed by RDA's to ascertain if the correlations between vegetation and the environmental variables were identical at all grain sizes. The data transformations were identical to those described above.

EFFECT OF INCREASING QUADRAT SIZE ON PLANT DENSITY

The average number of individuals per 100 cm² was plotted against the area of quadrat using contiguous quadrats (the eight 50 cm x 50 cm quadrats of the May sampling with the twenty-five 10 cm x 10 cm quadrats). Samples were generated by using the four corners of the 50 cm x 50 cm quadrats as the four starting points resulting in four sets of data for each of two 50 cm x 50 cm quadrats. The contiguous quadrats were laid down in such a way that they increased diagonally (1², 2², 3², 4², 5²), the 5² being identical in all four cases.

Results

RESULTS OF THE PCA USING 10 CM X 10 CM GRAIN SIZE AT THE TWO DIFFERENT EXTENTS

The PCA ordination diagram for the May 1992 data is shown in Fig. 3.1. The inclusion in the analysis of the covariables caused about 9% reduction in the total of the eigenvalues. The species-environment correlations were 0.279, 0.207, 0.229 and 0.255 for the first four axes. In addition the sum of the canonical eigen values was 0.070, a very low value.

Figure 3.2 shows the results of the PCA for the October data. The regression of the environmental variables revealed low correlation between the species and the environment (0.269, 0.281, 0.307 and 0.253 for axes 1-4). The canonical eigenvalue was 0.074.

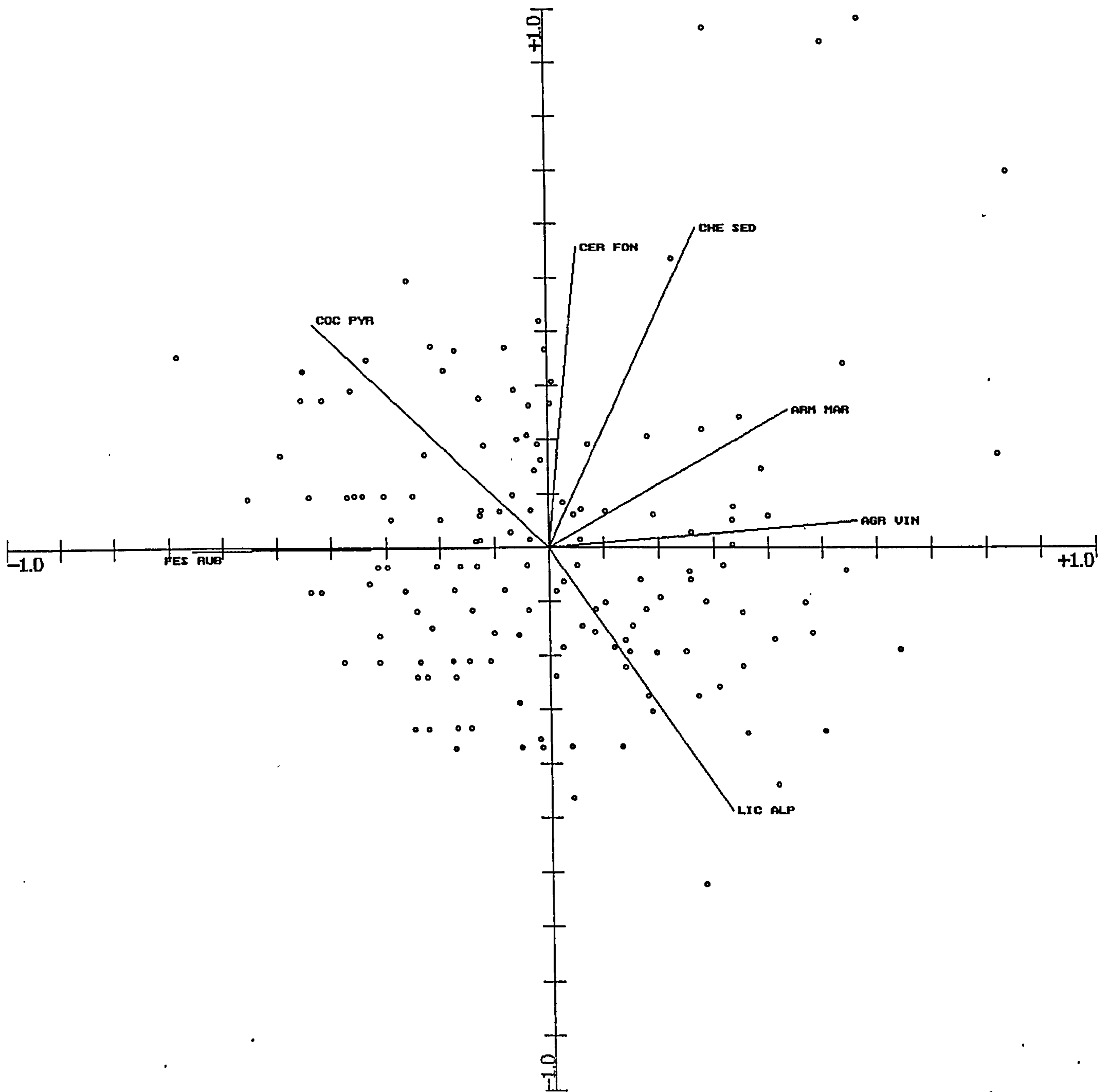


Fig 3.1 PCA (species centred and standardised by species norm) ordination diagram for the May 1992 data. The quadrat size was 10 cm x 10 cm (228 samples), and the extent of the sample area was 3 m x 3 m. AGR VIN, Agrostis vinealis; ARM MAR, Armeria maritima; CER FON, Cerastium fontanum ssp. scoticum; CHE SED, Minuartia sedoides; COC PYR, Cochlearia pyrenaica ssp. alpina; FES RUB, Festuca rubra; LYC ALP, Lychnis alpina.

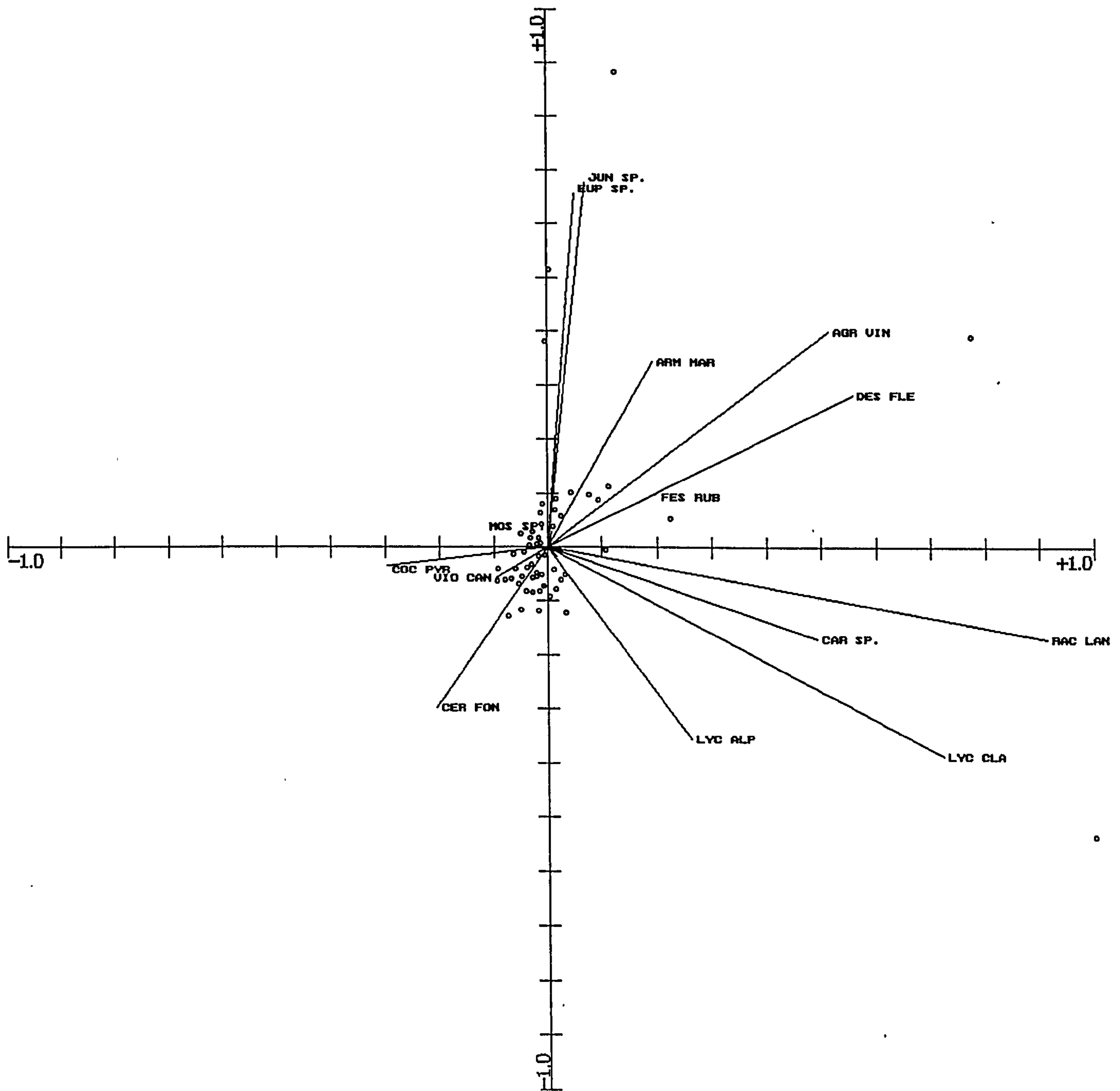


Fig 3.2 PCA (species centred and standardised by species norm) ordination diagram for the October 1992 data. The quadrat size was 10 cm x 10 cm (144 samples), and the extent of the sample area was 9 m x 7 m. Additional species abbreviations to those given for Fig. 3.1: CAR SP., Carex bigelowii; DES FLE, Deschampsia flexuosa; EUP SP., Euphrasia sp.; JUN SP., Juncus trifidus; MOS SP., unidentified moss, RAC LAN, Racomitrium lanuginosum, VIO CAN, Viola canina.

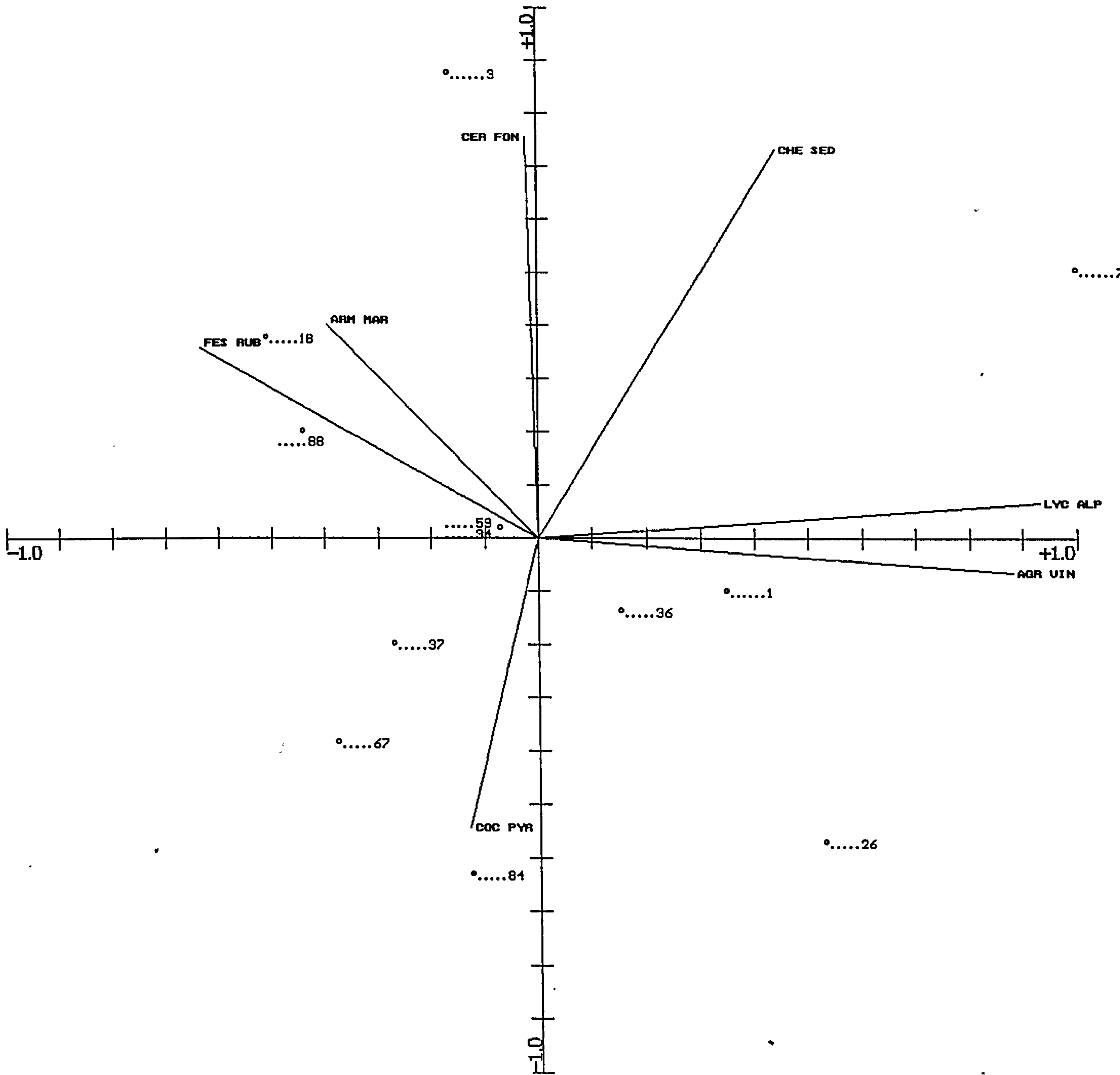


Fig 3.3 PCA (species centred and standardised by species norm) ordination diagram for the fourteen-sample set. The grain size was 10 cm x 10 cm (eight quadrats from the May set and six from the October set), and the extent of the sample area was 9 m x 7 m.

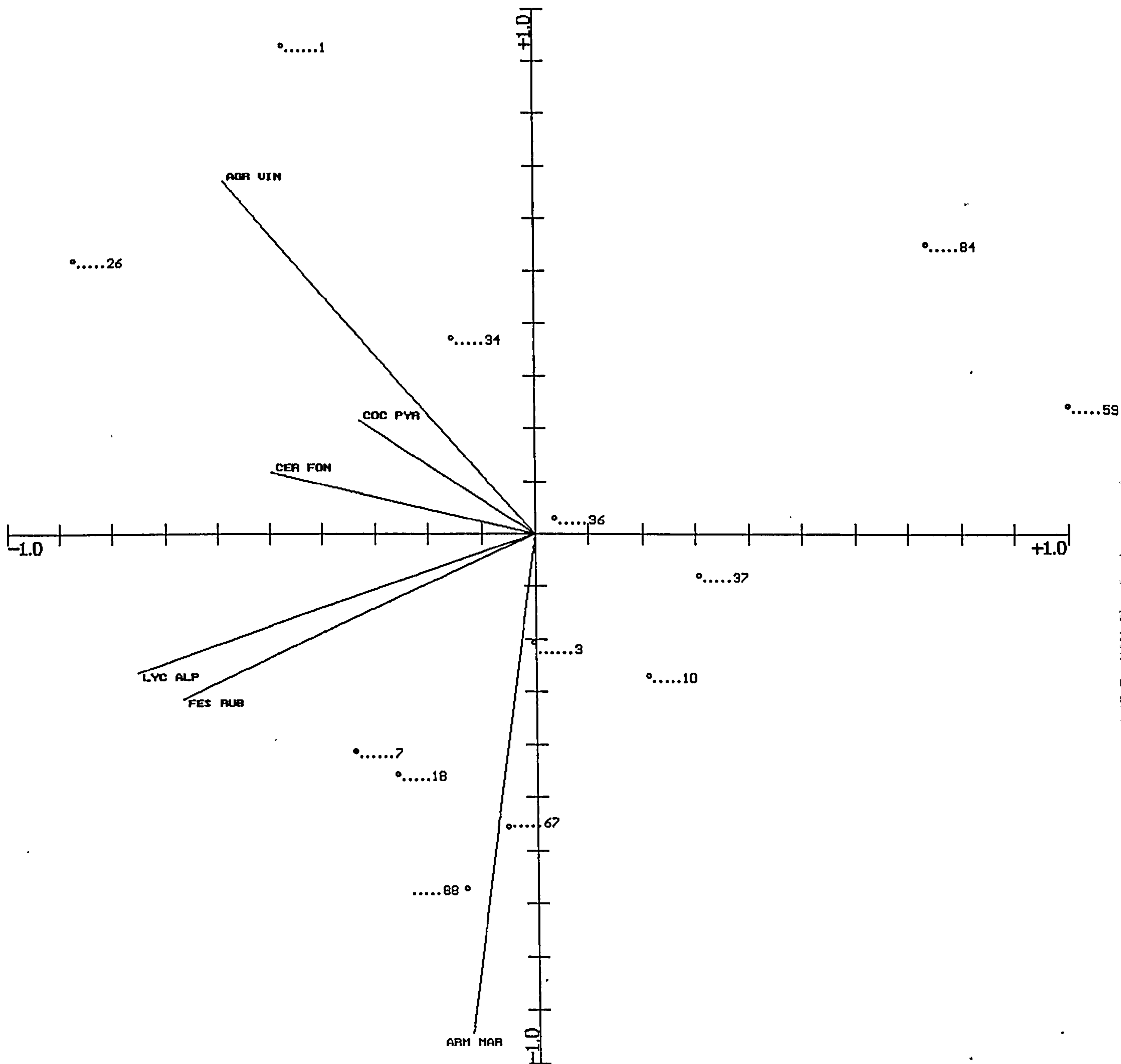


Fig 3.4 PCA (species centred and standardised by species norm) ordination diagram for the fourteen-sample set. The grain size was 20 cm x 20 cm (eight quadrats from the May set and six from the October set), and the extent of the sample area was 9 m x 7 m.

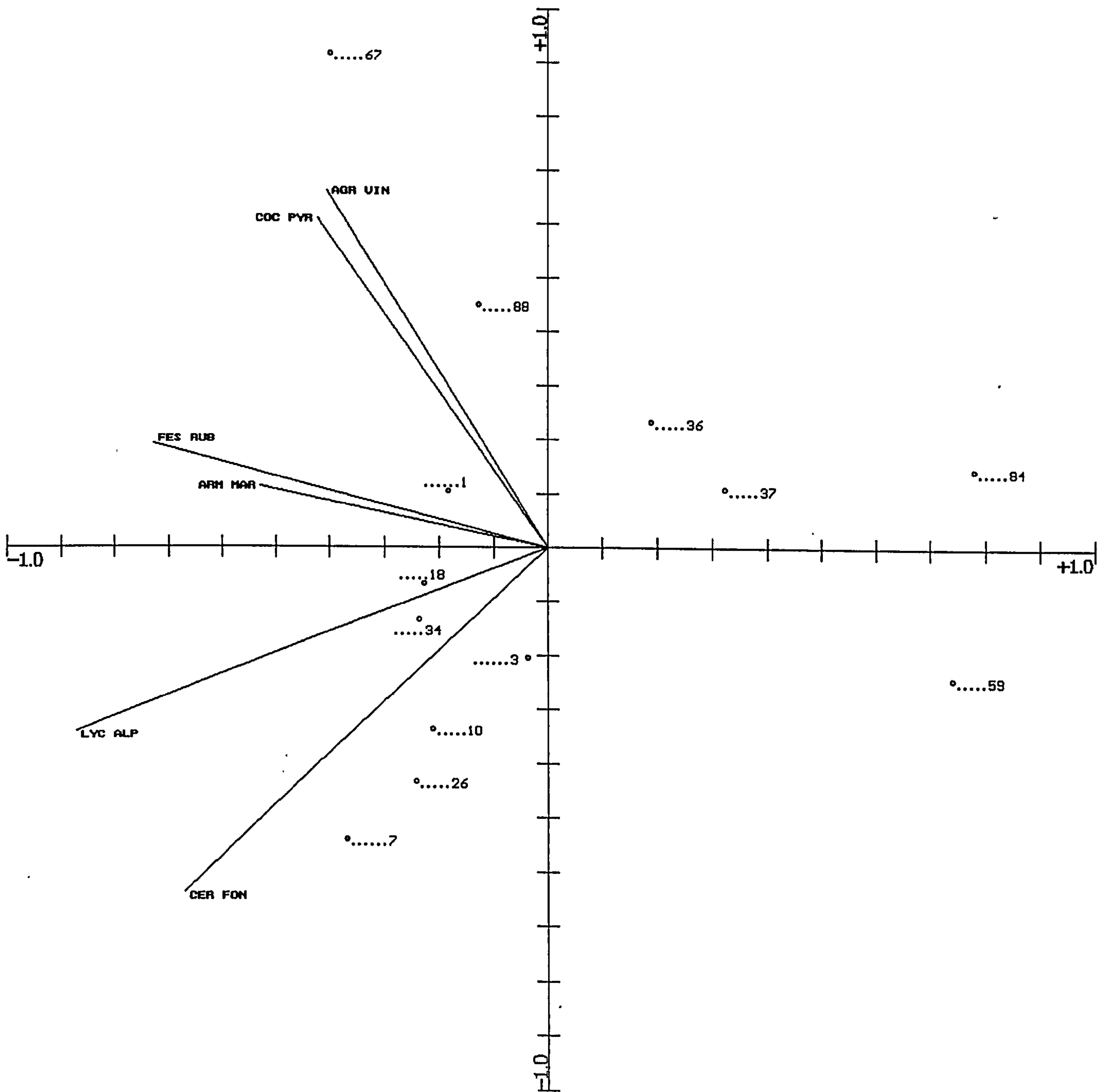


Fig 3.5 PCA (species centred and standardised by species norm) ordination diagram for the fourteen-sample set. The grain size was 30 cm x 30 cm (eight quadrats from the May set and six from the October set), and the extent of the sample area was 9 m x 7 m.

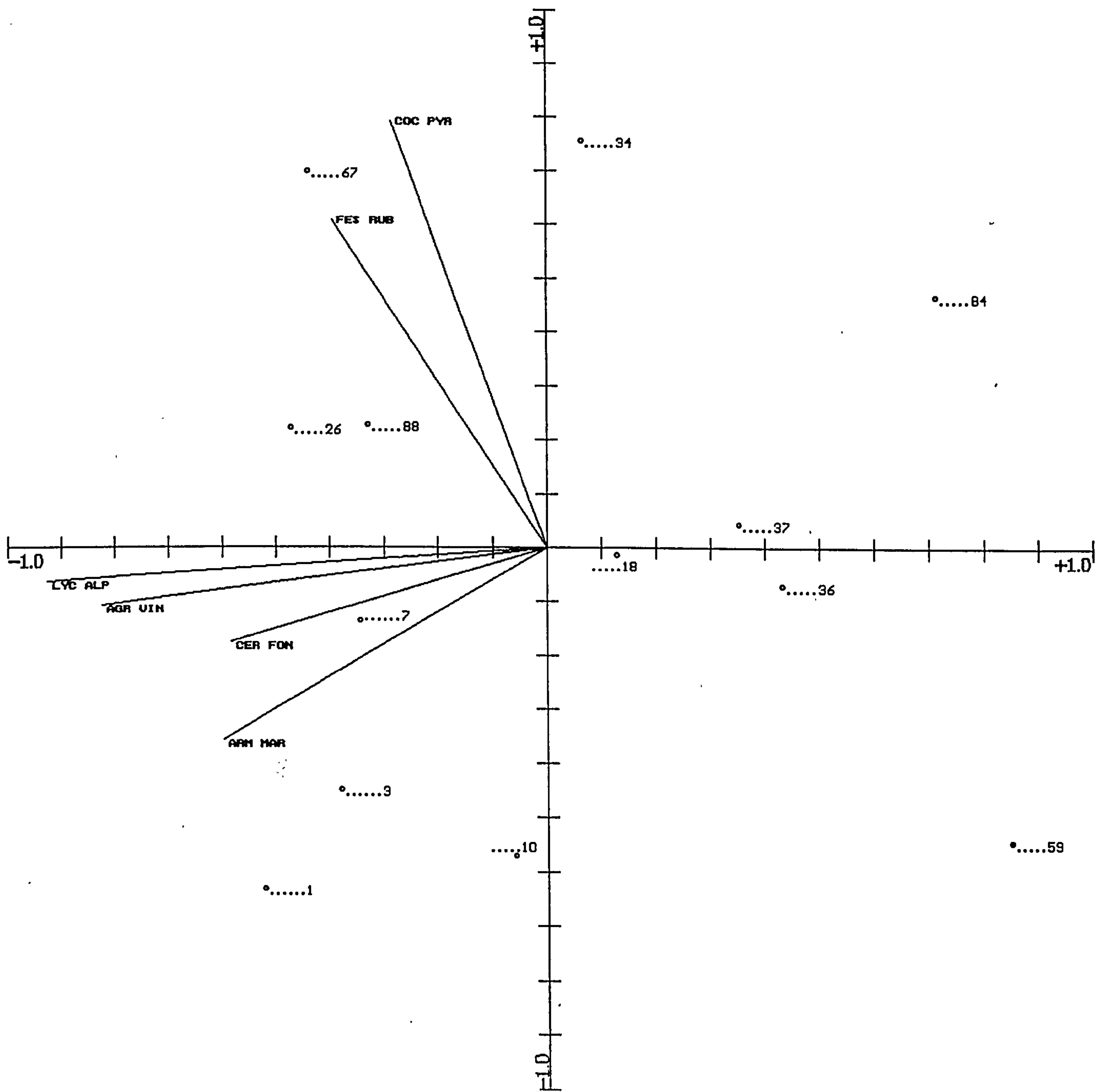


Fig 3.6 PCA (species centred and standardised by species norm) ordination diagram for the fourteen-sample set. The grain size was 40 cm x 40 cm (eight quadrats from the May set and six from the October set), and the extent of the sample area was 9 m x 7 m.

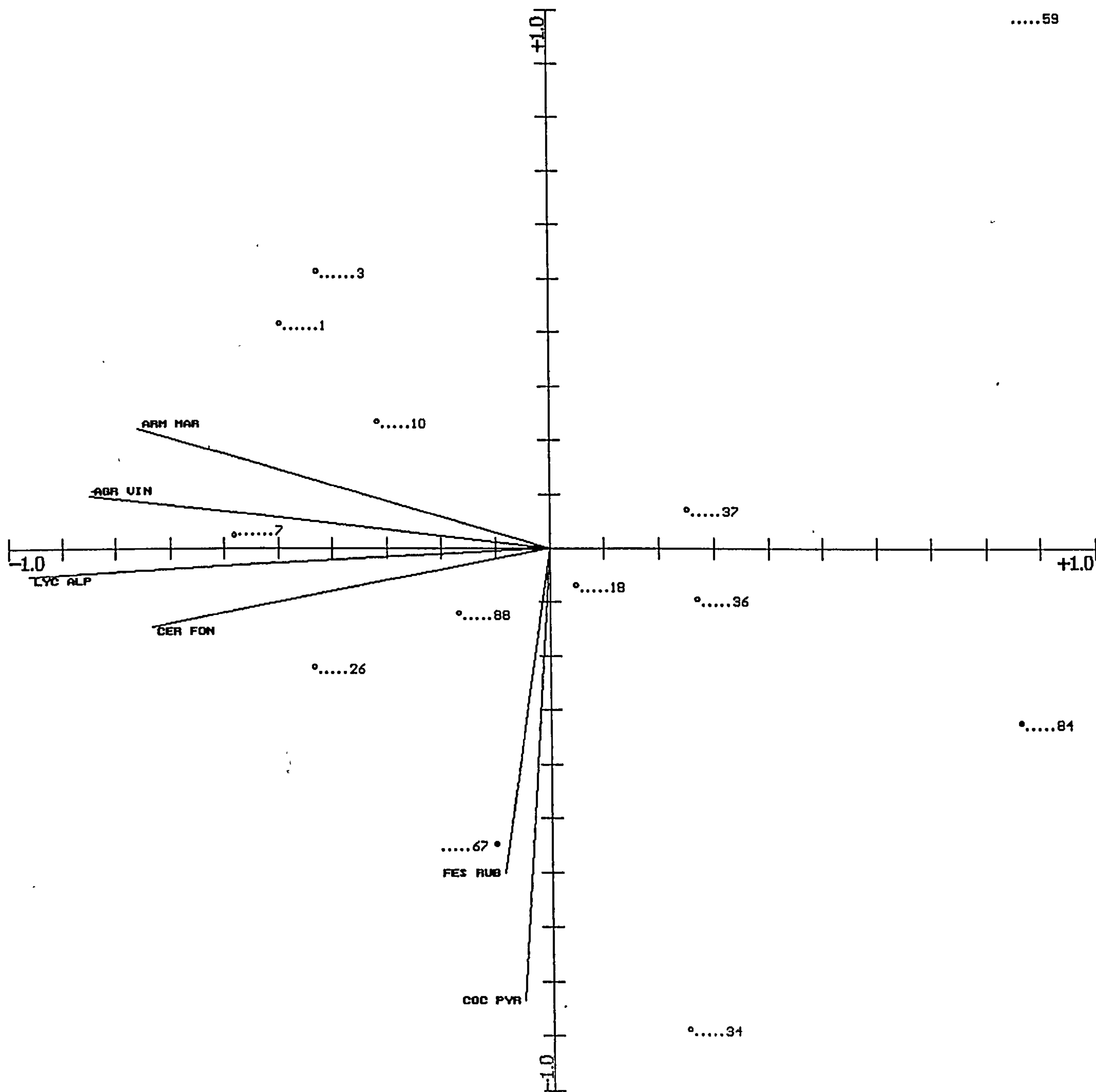


Fig 3.7 PCA (species centred and standardised by species norm) ordination diagram for the fourteen-sample set. The grain size was 50 cm x 50 cm (eight quadrats from the May set and six from the October set), and the extent of the sample area was 9 m x 7 m.

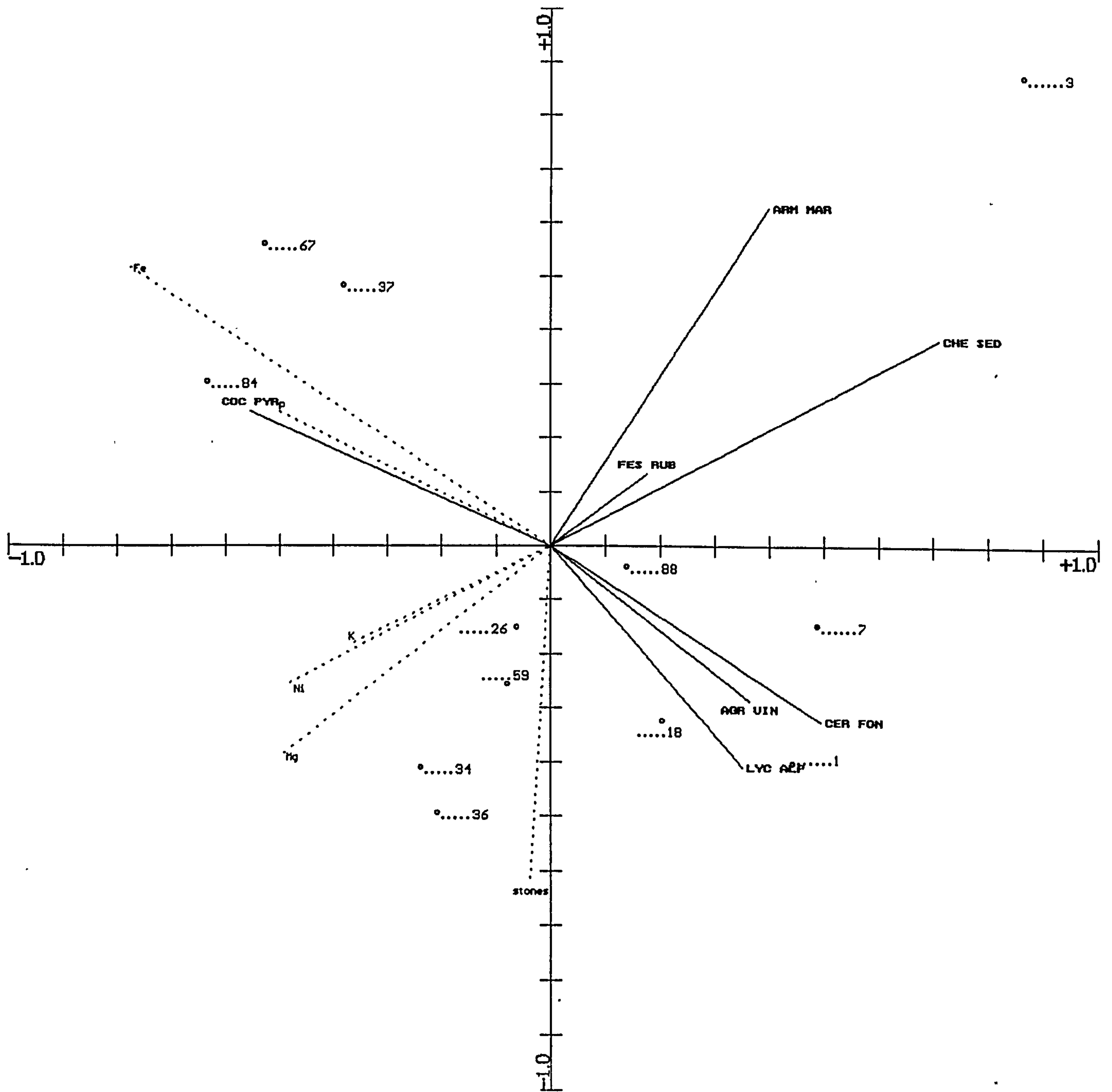


Fig 3.8 RDA (species centred and standardised by species norm) ordination diagram for the fourteen-sample set. The grain size was 10 cm x 10 cm (eight quadrats from the May set and six from the October set), and the extent of the sample area was 9 m x 7 m. The sum of constrained eigenvalues was 0.472.

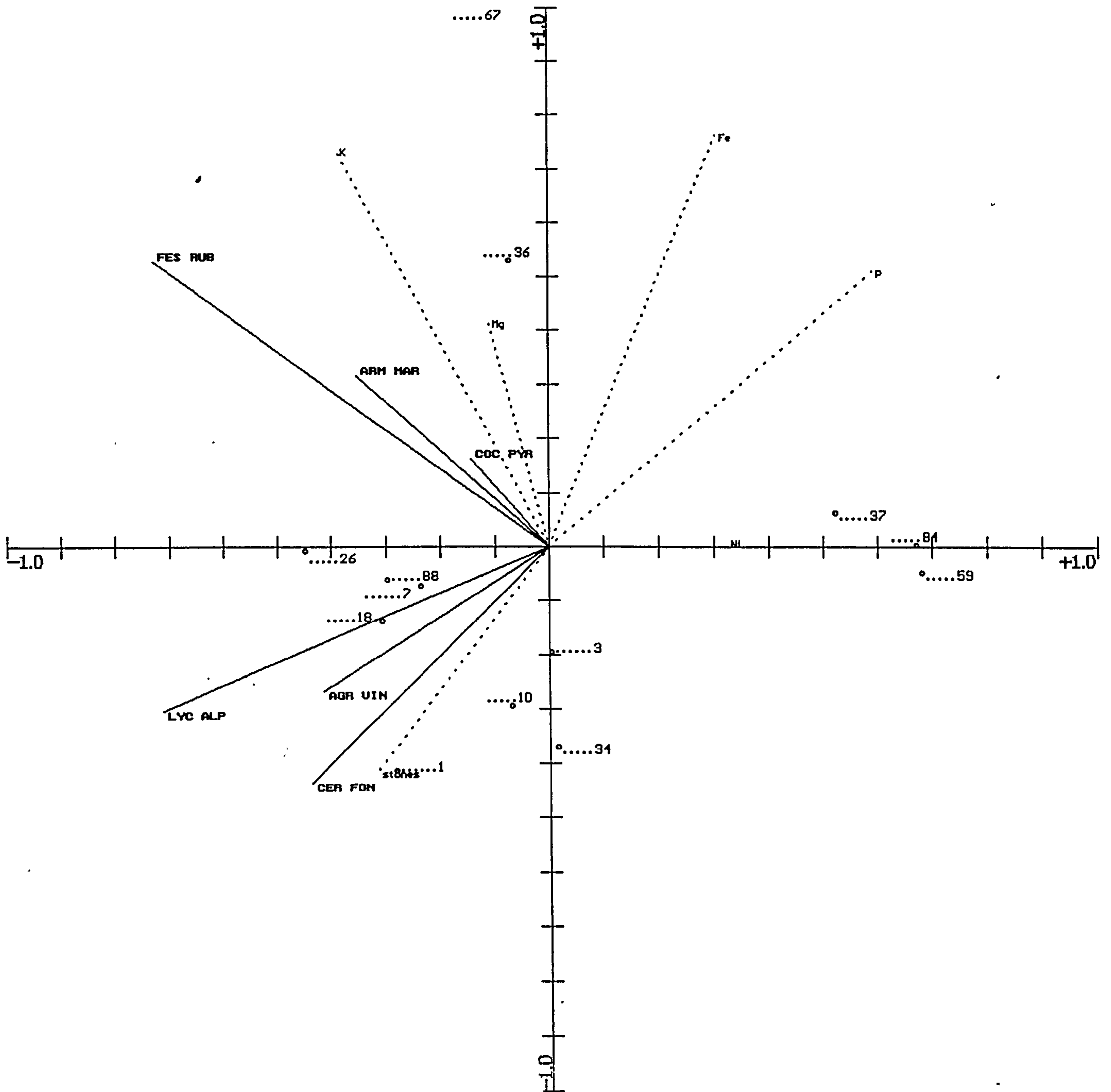


Fig 3.9 RDA (species centred and standardised by species norm) ordination diagram for the fourteen-sample set. The grain size was 20 cm x 20 cm (eight quadrats from the May set and six from the October set), and the extent of the sample area was 9 m x 7 m. The sum of constrained eigenvalues was 0.557. The environmental variables for the six quadrat from the October sample set are estimates obtained by punctual kriging. Numbers refer to sub-plots (quadrats).

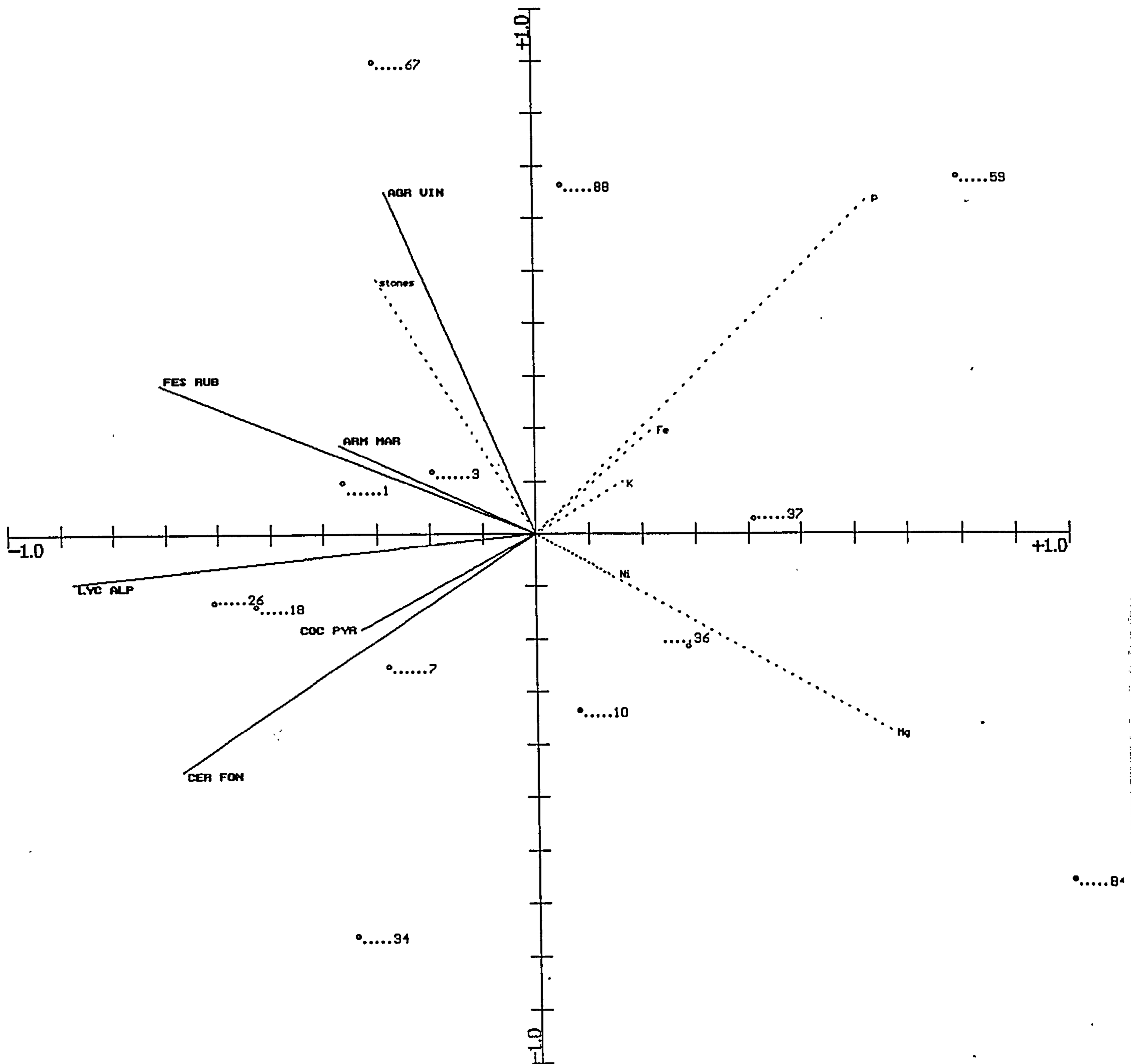


Fig 3.10 RDA (species centred and standardised by species norm) ordination diagram for the fourteen-sample set. The grain size was 30 cm x 30 cm (eight quadrats from the May set and six from the October set), and the extent of the sample area was 9 m x 7 m. The sum of constrained eigenvalues was 0.664. The environmental variables for the six quadrat from the October sample set are estimates obtained by punctual kriging. Numbers refer to sub-plots (quadrats).

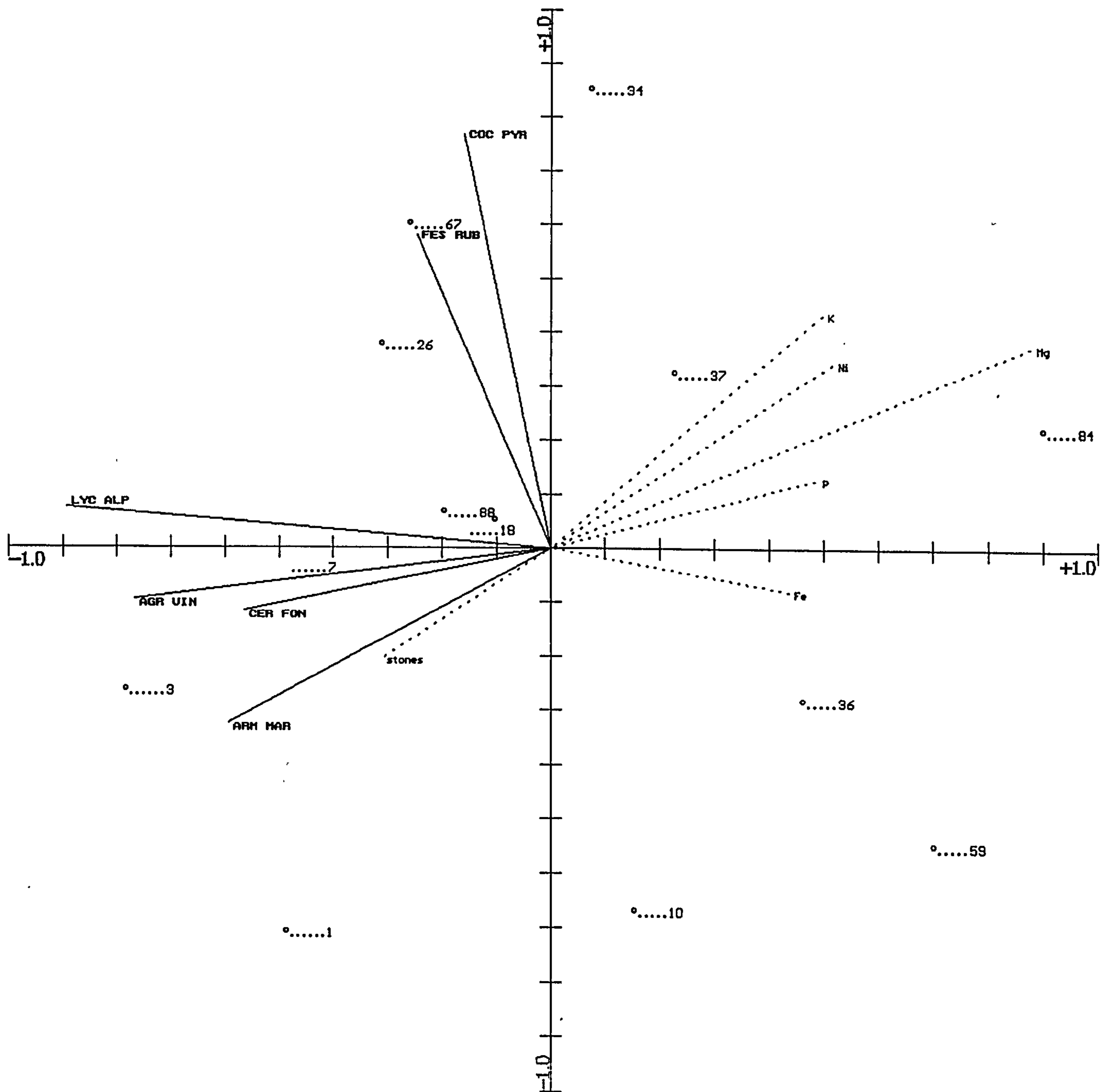


Fig 3.11 RDA (species centred and standardised by species norm) ordination diagram for the fourteen-sample set. The grain size was 40 cm x 40 cm (eight quadrats from the May set and six from the October set), and the extent of the sample area was 9 m x 7 m. The sum of constrained eigenvalues was 0.758. The environmental variables for the six quadrat from the October sample set are estimates obtained by punctual kriging. Numbers refer to sub-plots (quadrats).

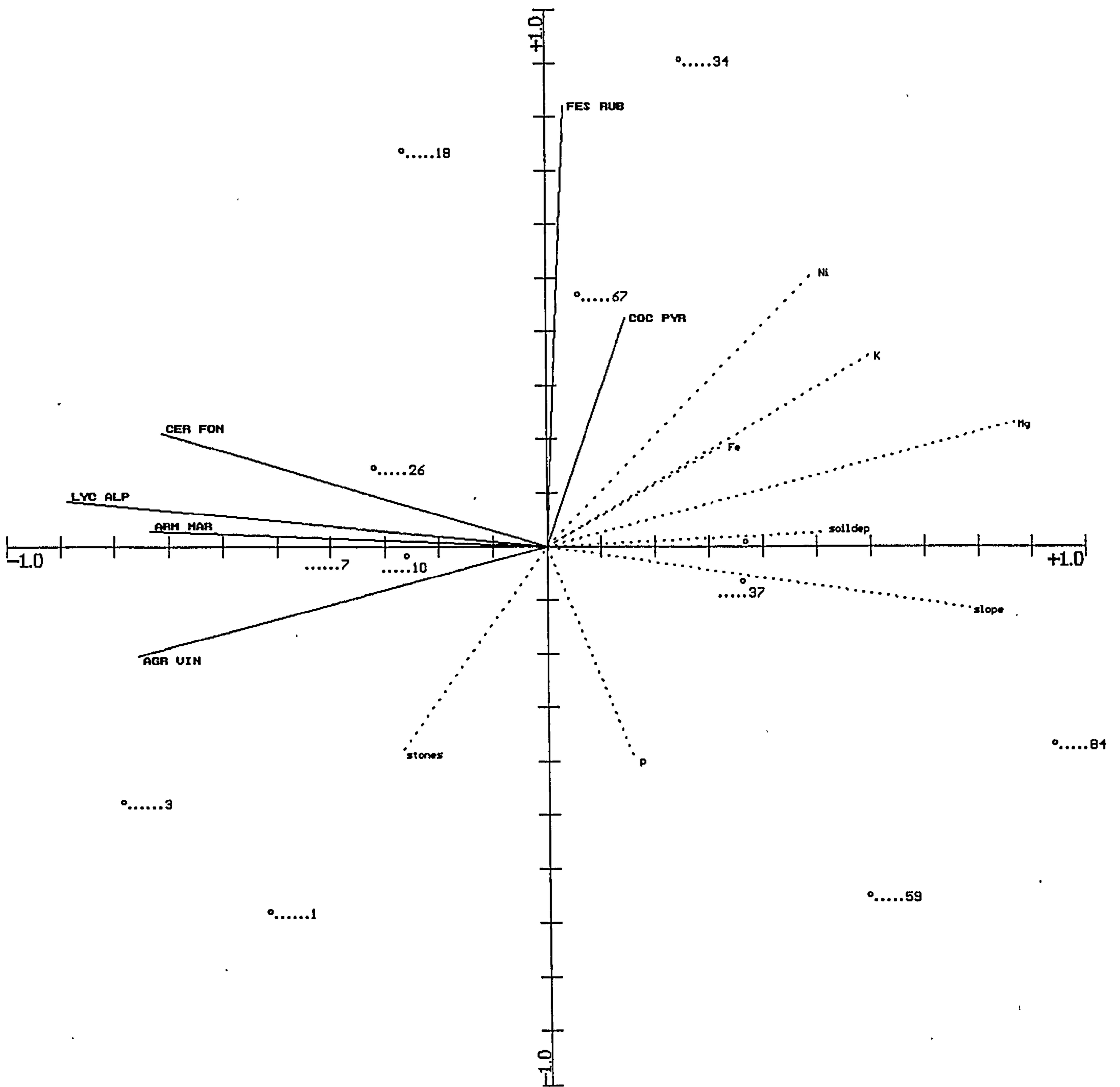


Fig 3.12 RDA (species centred and standardised by species norm) ordination diagram for the fourteen-sample set. The grain size was 50 cm x 50 cm (eight quadrats from the May set and six from the October set), and the extent of the sample area was 9 m x 7 m. The sum of constrained eigenvalues was 0.800. The environmental variables for the six quadrat from the October sample set are estimates obtained by punctual kriging. Numbers refer to sub-plots (quadrats).

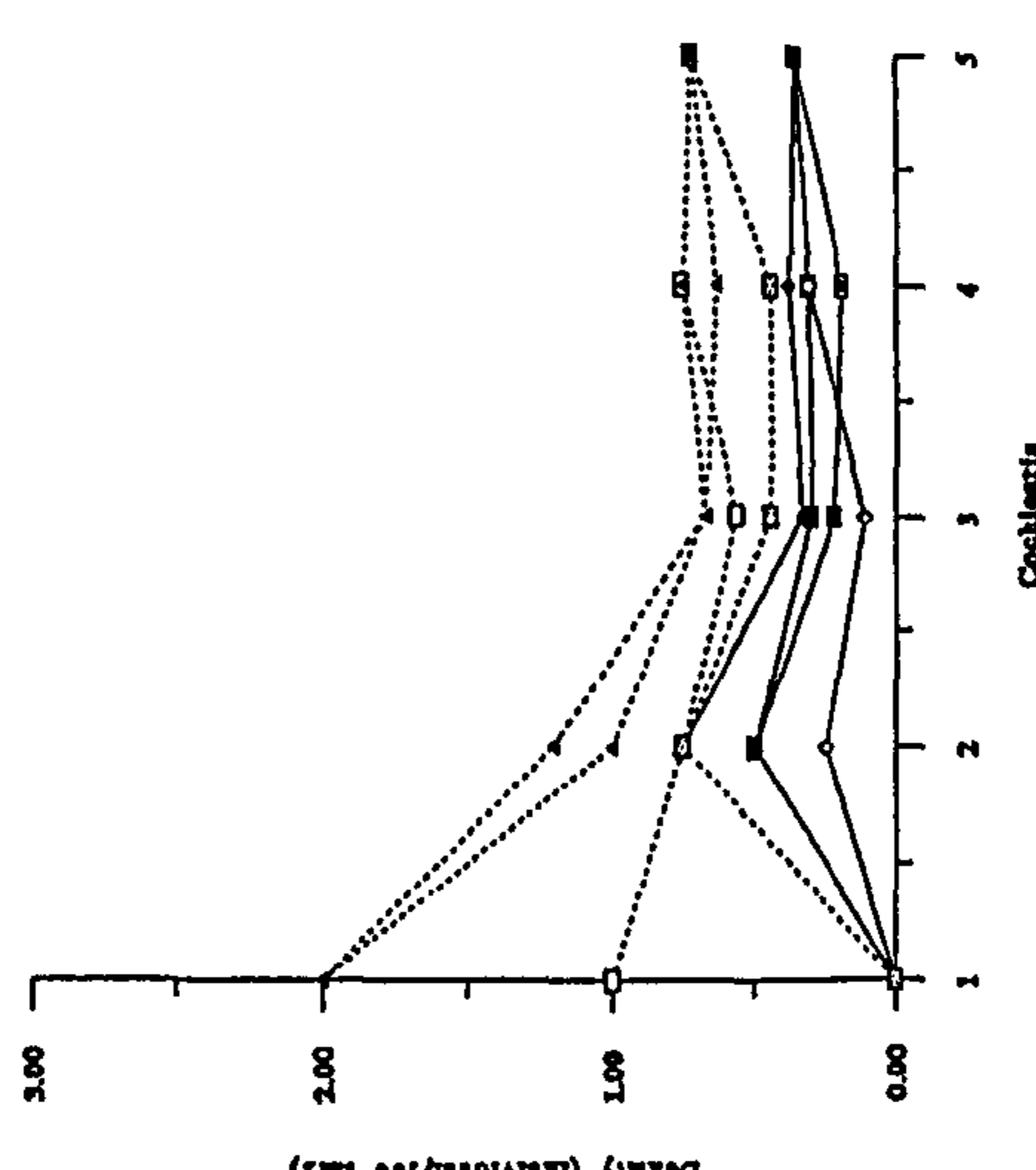
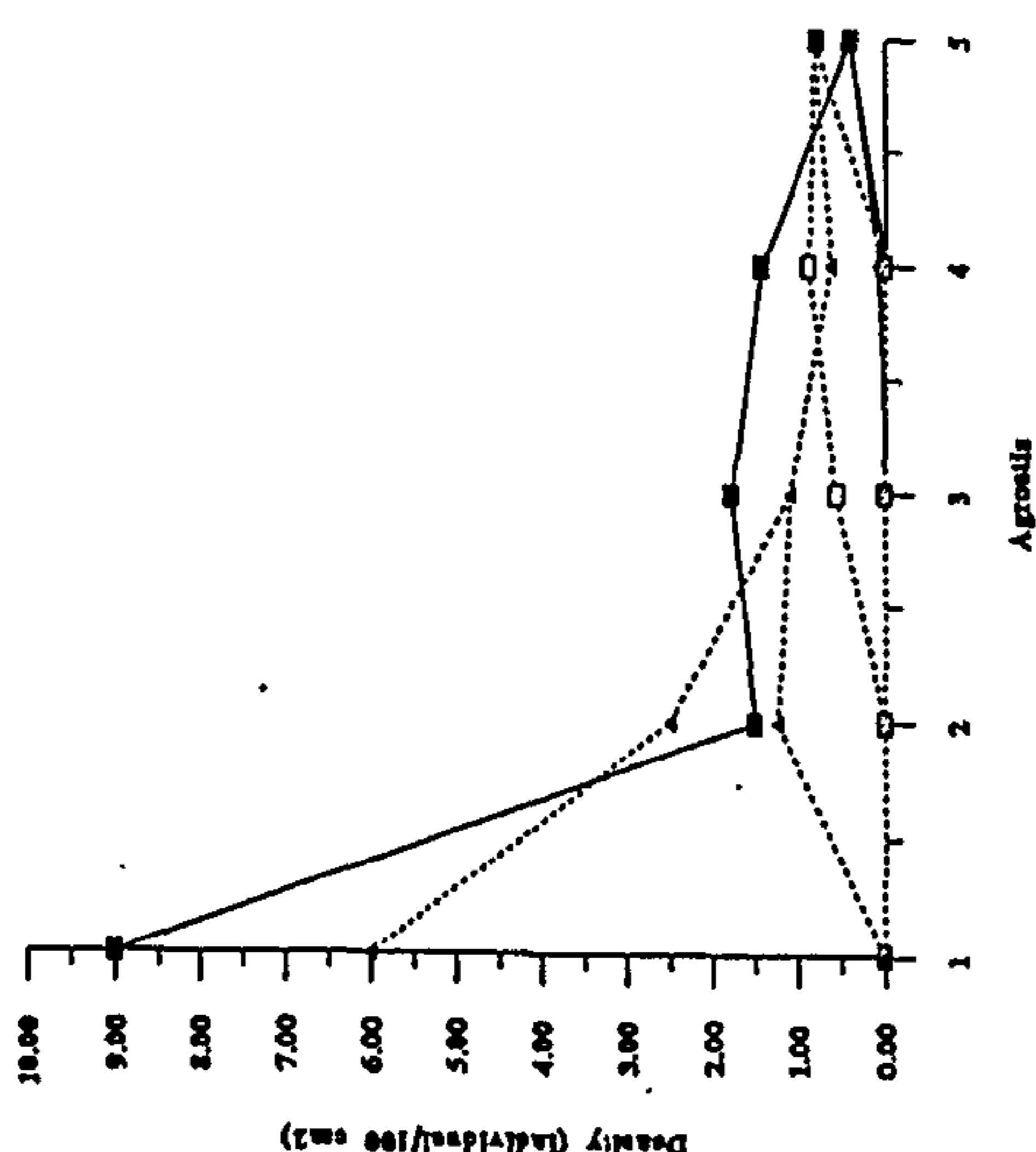
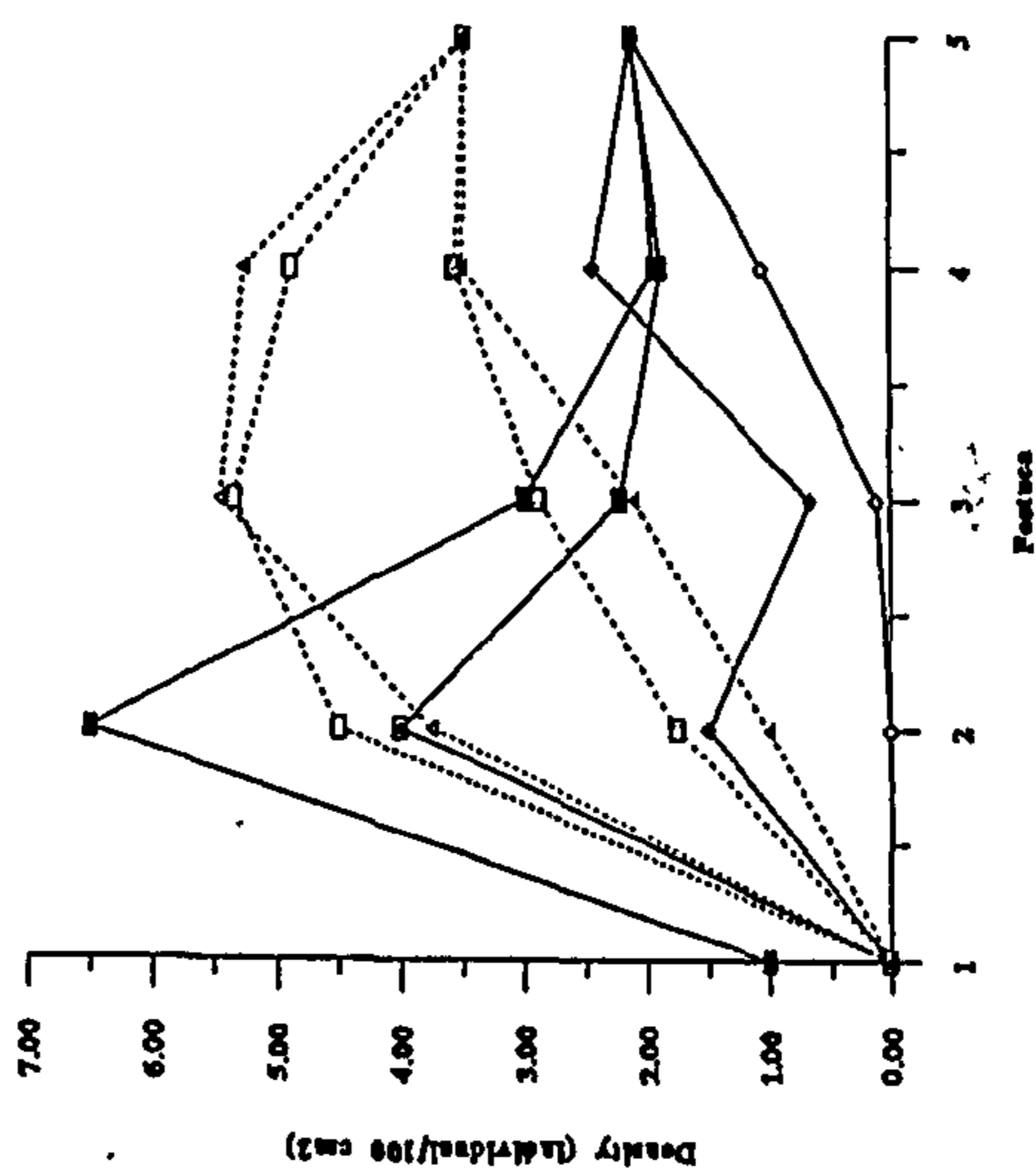
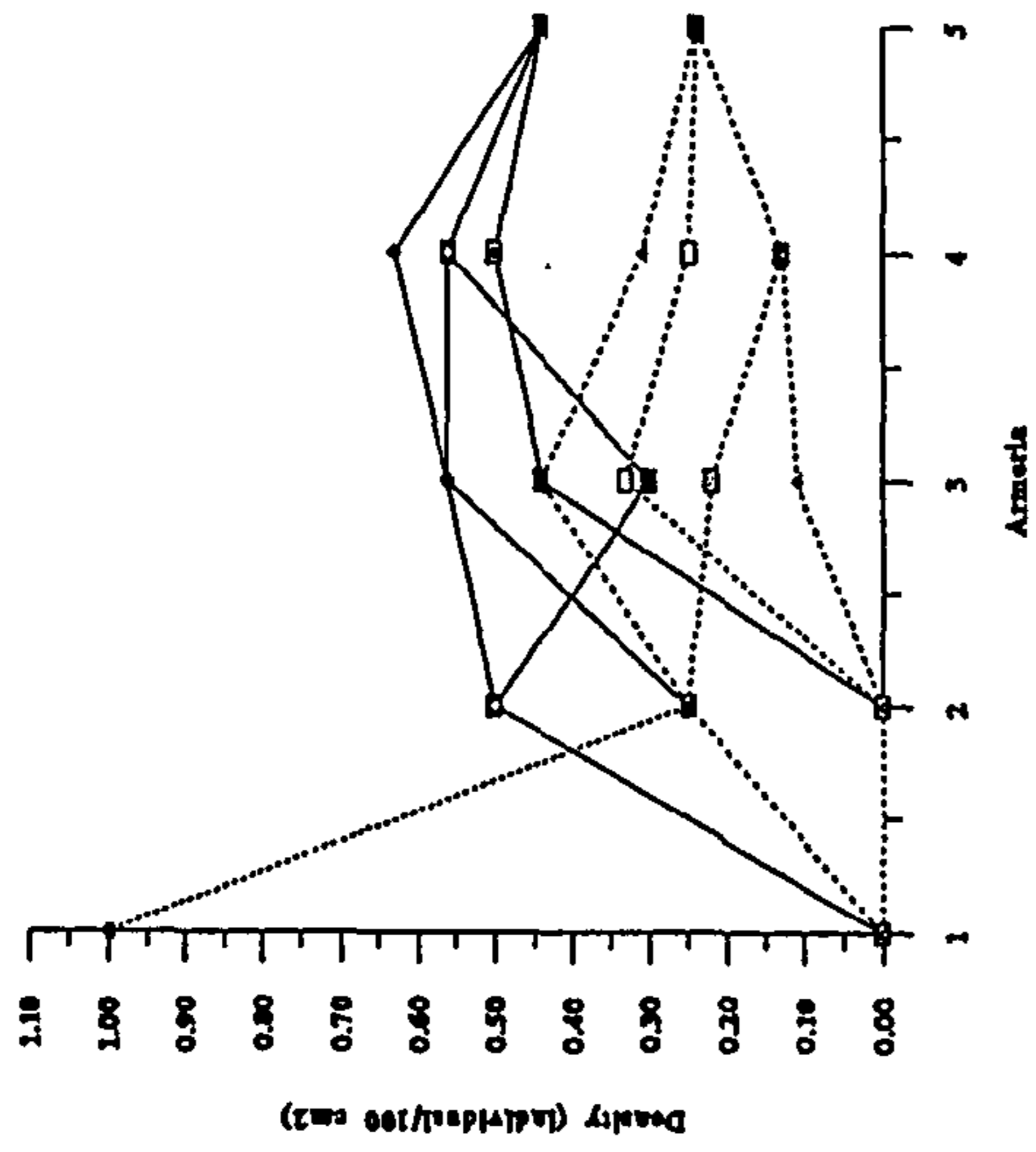
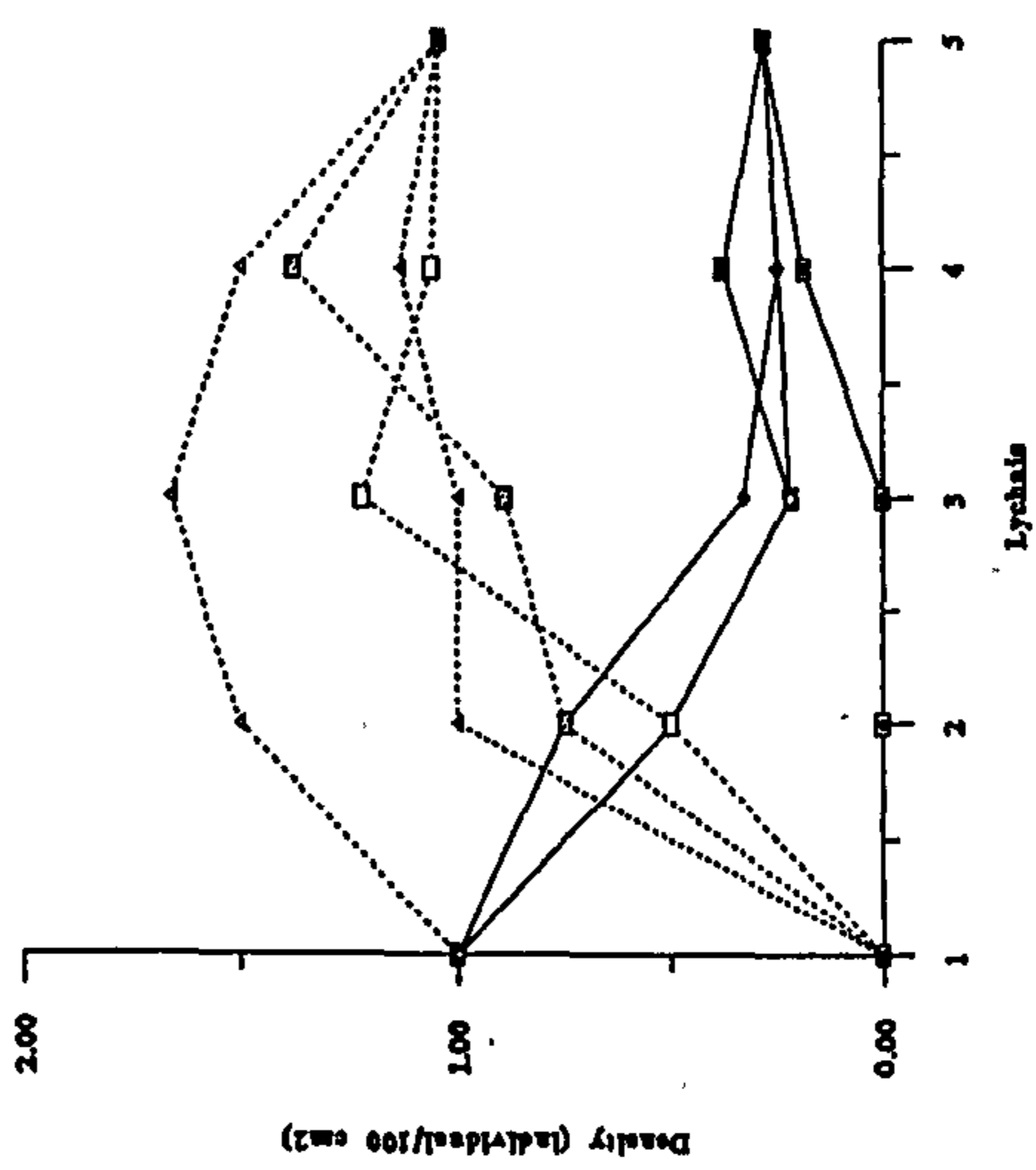
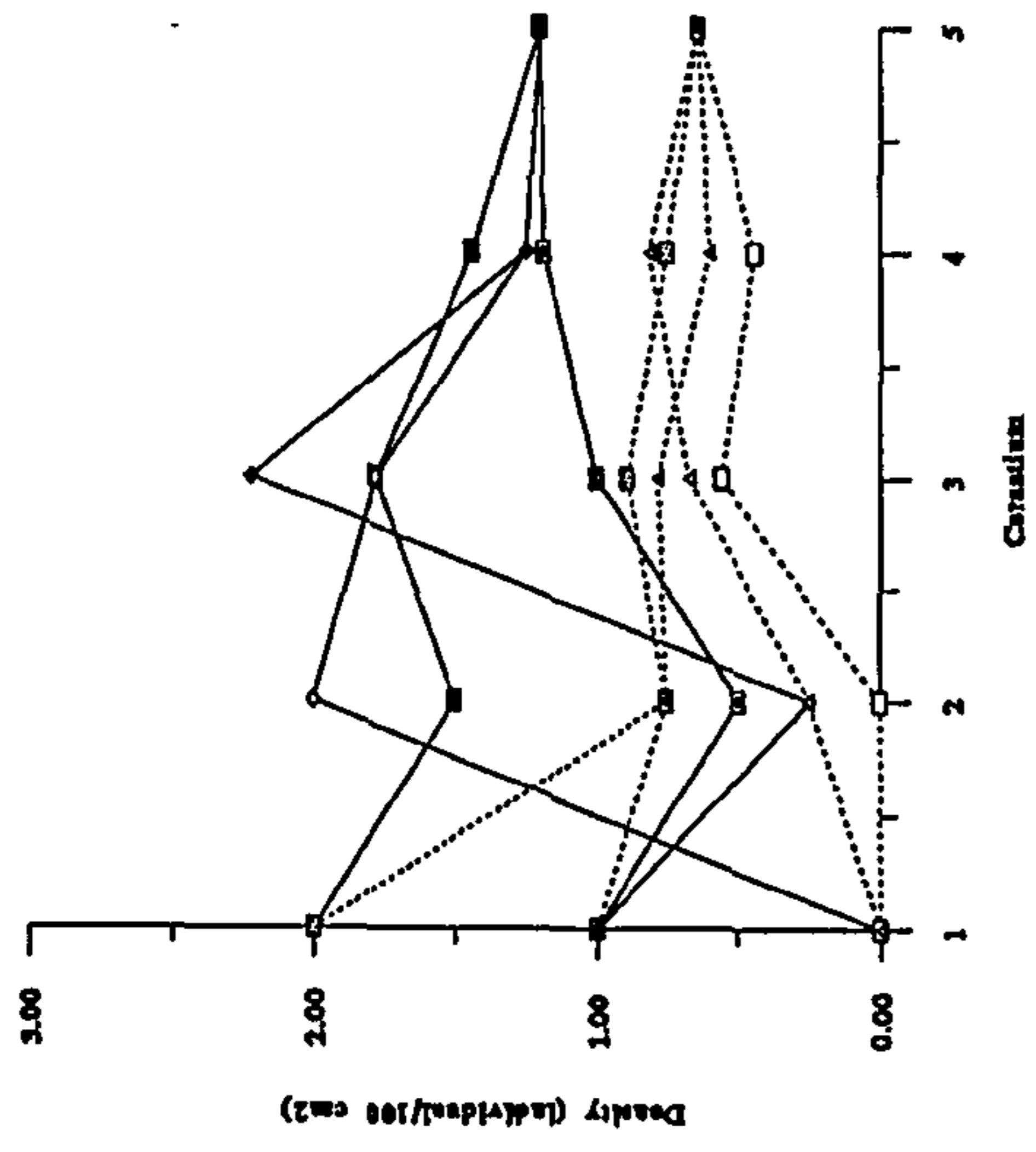


Fig 3.13 The effect of increasing quadrat size (1, 100 cm²; 2, 400 cm²; 3, 900 cm²; 4, 1600 cm²; 5, 2500 cm²) on the density of Agrostis vinealis, Armeria maritima, Cerastium fontanum ssp. alpina, Cochlearia pyrenaica ssp. alpina, Festuca rubra, and Lychnis alpina.

VEGETATION-ENVIRONMENT CORRELATIONS USING FIVE GRAIN SIZES

Pattern at the 10 cm x 10 cm to 40 cm x 40 cm grain size

Figures 3.3-3.7 show the PCA diagrams for the 10 cm x 10 cm, 20 cm x 20 cm, 30 cm x 30 cm, 40 cm x 40 cm and 50 cm x 50 cm quadrats. The relative position of the species along the first two axes showed high variation with increasing quadrat size showing convergence in the 40 cm x 40 cm to that in the 50 cm x 50 cm.

The use of constrained ordination resulted in dissimilar results from that of the PCA's at the two smallest grain sizes (10 cm x 10 cm and 20 cm x 20 cm) (Figs. 3.3-3.4 v. Figs. 3.8-3.9). This was the result of the low total of canonical eigenvalues (0.472 and 0.557) relative to those in the PCA's. The increase in the canonical eigenvalues at larger grain sizes (0.664, 0.758, 0.733) was reflected in better agreement between the PCA and RDA results. The investigation of correlations between vegetation and environmental variables showed that there was no consistent pattern over the scale considered (Figs. 3.8-3.12). A relatively good agreement was only found between the two largest grain sizes.

Pattern at the 50 cm x 50 cm grain size

A high level of species-environment correlations was found for the first four PCA axes (0.944, 0.888, 0.953 and 0.882). The results of the subsequent RDA are displayed in the triplot for the fourteen 50 cm x 50 cm quadrats in Figs. 3.7 and 3.12. Using constrained ordination did not decrease the sum of the eigenvalues to a large extent (1.000 v. 0.733

and 1.000 v. 0.800 when slope was also included), which indicates that the environmental variables included in the analysis were satisfactorily explaining the underlying gradients. Mg^{2+} , slope, and K^+ were most highly correlated with axis 1, and Ni^{2+} positively, and stoniness and $H_2PO_4^-$ negatively with axis 2. Agrostis, Armeria, Cerastium and Lychnis had high negative scores on axis 1 showing a negative relationship with slope, Mg^{2+} , K^+ , soil depth, and (weakly) with stoniness. Festuca showed no significant correlations with variables for axis 1 and stoniness seems to have had the largest effect on its distribution. Cochlearia showed the highest correlation with stoniness (negative), and with Ni^{2+} , K^+ , iron and Mg^{2+} (positive) in decreasing order.

EFFECT OF INCREASING QUADRAT SIZE ON PLANT DENSITY

Figure 13 shows the effect of increasing quadrat size on density. The two grass species Agrostis and Festuca showed a very unstable average density value with increasing quadrat size and no convergence to a final value at 50 cm x 50 cm. The two multiple-rosette forming species Armeria and Lychnis also required the size of 40 cm x 40 cm to give a broad agreement with the densities at the final quadrat size investigated. Cerastium and Cochlearia on the other hand showed a low amplitude of variation after reaching the 30 cm x 30 cm size.

Discussion

There were no differences between the environment-vegetation correlations with increased extent of sampling. This agreed with Reed *et al.*'s finding in a forest (1993). The data from the fine-grained sampling (10 cm x 10 cm) at both area sizes showed a

very low correlation between vegetation and the environment. At first sight this may be taken to indicate that the species distribution was not related to the environmental variables measured. In that case the observed pattern might be a result of growth form and restricted seed dispersal and vegetative growth. If that were so, one would expect such an explanation to hold at any scale of observation and regional differences between sub-plots would not be apparent. The PCA on the May data, allowing for the block effect, gave a similar result to that where all quadrats were treated as independent samples. However, it could clearly be demonstrated that there were significant differences between sub-plots with increasing availability of the cations down-slope (Table 2.5) and there were grounds to expect this difference to be reflected in species composition (provided that the differences in 'the composite environmental variable' are large enough to induce a species-scale response, Greig-Smith 1983). When the environmental factors were correlated with the vegetation data at the 10 cm x 10 cm resolution the regional (between sub-plot) differences were not obvious. The very low values obtained for the RDA eigenvalues were an indication of the poor correlations between vegetation and the environment factors investigated at this grain size. It is noteworthy that when the analysis was repeated at the same resolution on a fourteen-sample sub-set, representing the fourteen sub-plots, better agreement was found between the eigenvalues for the unconstrained and constrained ordination (1.000 and 0.472) than was the case when the full data set were analysed (corresponding eigenvalues were 1.000 and 0.070). A word of caution is appropriate here on the limitations of this type of analysis using a small number of samples only.

Was the quadrat size of 10 cm x 10 cm appropriate for this analysis? The results of the exhaustive sampling of the eight 50 cm x 50 cm sub-plots revealed a high point-to-point

variation for both vegetation and environment. The analysis of the effect of using increasing successive contiguous quadrat sizes for vegetation sampling showed that there was too high a chance of a hit, or miss for the 10 cm x 10 cm quadrats to represent an 'average' vegetation (Fig. 3.13) It could be seen that the convergence to an average density value required at least a 40 cm x 40 cm quadrat size for most of the species, especially the grasses, reflecting their vegetative spread, or clumped growth due to restricted seed dispersal as in Lychnis for example.

Samples of 10 cm x 10 cm had seemed appropriate because they contained a range of numbers of individuals from zero to many with various combinations of species. Moreover it is questionable if quadrats of this size were relevant for plants as a whole. Plants are likely to extend their root system beyond the area of their aerial parts, especially in shallow, nutrient-poor soil. They are likely to exploit a range of 'microhabitats' (Fitter & Hay 1987), which may have much environmental variation at the micro-scale. This characteristic of plants has largely been overlooked at scale selection for pattern studies. The large extent of plant root systems would support the use of regional estimates for gradient analysis rather than individual measured values since regional estimates are based on averaging neighbouring values using an algorithm which takes into account overall trends with distance and direction. Successive analyses on increasingly larger vegetation units to detect pattern and environment-vegetation correlation should then use environmental variables estimated at correspondingly coarser resolutions to reflect more of an average environment. The use of environmental variables estimated in the above way may, however, mask local extremes which may have important effects on plant establishment, or survival.

We must be aware of Wiens' (1989) cautioning words: 'If we study a system at an

inappropriate scale, we may not detect its actual dynamics and patterns but may instead identify patterns that are artefacts of scale. Because we are clever at devising explanations of what we see, we may think we understand the system when we have not even observed it correctly'.

The higher correlation between environment and vegetation with increasing grain size was in concert with Reed et al.'s report (1993). There was, however, an inconsistency in the results for correlations between environmental factors and vegetation obtained by using different grain sizes (also found by Reed et al. 1993). This indicated that different factors (and possibly processes) were of importance at different scales at MK1. It is generally accepted that when one makes observations using a specific grain size at a given extent, the validity of deductions is also limited. The use of different grain sizes in this study demonstrated the level of uncertainty involved. It is reasonable to conclude that, having examined by various techniques the patterning of vegetation and soil variation at MK1 (variograms in Chapter II. and levels of environment-vegetation correlations, this Chapter), that a minimum grain size of 40 cm x 40 cm is to be recommended for a hypothesis-generating analysis. There are however constraints as to the extent because of the actual physical sizes of open areas of skeletal soil.

There are aspects, for example the environmental-gradient induced genetic differentiation in species, which fall outside the scope of the present study, but which may be of great importance for some rarities at the site such as Cerastium and Lychnis.

Within the range of species-scale response, small-scale patterning of the environment may also lead to the development and maintenance of genetically diverse populations (Lechowicz & Bell 1991), or genetic differentiation at the genotype level and differential physiological responses (Monson et al. 1992).

In view of the above discussion on grain size and the scale of spatial dependence exhibited by the plant species (Chapter II) one can most reliably accept the results of the ordination carried out on the 50 cm x 50 cm quadrat data. The environment-species correlations suggested different factors as possible causes for species distribution: it is hypothesised that Festuca is indifferent to MK1 soil chemistry; Agrostis, Cerastium, and Lychnis prefer low water tables and are magnesium avoiders; and Cochlearia is tolerant of high water table and high concentrations of cations, especially nickel. The responses to solution magnesium and nickel by Cerastium fontanum ssp. scoticum, Cochlearia pyrenaica ssp. alpina and Festuca rubra will be tested in Chapter IV; and the impacts of various iron and nickel availabilities for Cochlearia pyrenaica ssp. alpina will be investigated in Chapter V.

Chapter IV

Comparative growth experiments using ultramafic and non-ultramafic races of Cerastium fontanum, Cochlearia pyrenaica and Festuca rubra

Introduction

Magnesium toxicity has been considered as the major chemical factor in accounting for the unusual floristic features of the vegetation of the ultramafic outcrops near Meikle Kilrannoch (Proctor 1970, 1971). Proctor & McGowan (1976), using oat (Avena sativa) plants in conventional culture media showed that magnesium toxicity may be reduced by nickel, an element which is at relatively high concentrations in many ultramafic soils including Meikle Kilrannoch. Later, using Festuca rubra clones grown in water culture solutions based on soil solution extracts, Johnston & Proctor (1981) showed that nickel as well as magnesium was at potentially toxic concentrations, and in contrast to Proctor & McGowan (1976), provided evidence of positive interactive toxic effects of the two elements.

One criticism of the work of Proctor & McGowan (1976) and Johnston & Proctor (1981) was their use of FeEDTA as an iron source. Several authors have recently cautioned that interferences in solution Fe-chemistry may lead to erroneous conclusions and suggested that FeEDDHA would be a better iron source than FeEDTA (Chaney & Bell 1987). Computer programs have been developed to predict the likely ion speciation

and equilibria in solution by using calculated stability constants (Halvorson & Lindsay 1972, Sposito & Mattigold 1980).

Iron chelates have long been used as an iron source in hydroponic systems. When Fe-chelates are added to nutrient solutions some of the Fe^{3+} is displaced by other cations and hydrous-ferric oxide is precipitated. The efficacy of different chelates to prevent the precipitation of iron depends on their stability constant values, which in turn are often influenced by solution pH (Lindsay 1979). A series of experiments were conducted to characterise chelates available for plant research (Halvorson & Lindsay 1972, Chaney 1988).

The implications of iron displacement are many-fold. Through precipitation iron supply may reach a level which limits plant growth. When the hydrous-ferric oxide precipitates on the root surface it may adsorb phosphorus and other nutrients (Chaney & Bell 1987) and introduce an artefact in subsequent analytical work. In metal toxicity studies, metal-chelates with high stability constants may be formed and the availability of the chelated metals reduced. Halvorson & Lindsay (1977) give an instance where zinc availability was reduced by chelation of the element. Recent work, where GEOCHEM was used to assess the chemistry of the culture solutions used by Johnston & Proctor (1981) and Proctor & McGowan (1976), suggested that their use of NaFeEDTA might have affected their results through possibly reducing both iron and maybe nickel availability (Proctor & Nagy 1992). One interest for the present study was if the type of iron-chelate used had significant effects on plant growth in different Mg/Ni treatments. Three species which are important in the make-up of the vegetation on the skeletal soil at the Meikle Kilrannoch ultramafic outcrops were selected: Cerastium fontanum ssp. scoticum, Cochlearia pyrenaica ssp. alpina and Festuca rubra for use in two

experiments.

Experiment 1 compared the dry matter production and the uptake of iron and nickel by Cochlearia species grown in solutions with FeEDTA and FeEDDHA to establish if the choice of the iron-chelate significantly influenced plant growth in different Mg/Ni treatments and also if the use of FeEDTA in earlier work (Proctor & McGowan 1976, Johnston & Proctor 1981) may have resulted in erroneous conclusions.

Experiment 2 compared the effects of magnesium and nickel alone and in combination on the growth of ultramafic and non-ultramafic species pairs of each of Cerastium fontanum (Caryophyllaceae), Cochlearia pyrenaica (Brassicaceae) and Festuca rubra (Poaceae). The main aims of experiment 2 were (a) to test the hypothesis that growth responses to high concentrations of magnesium and nickel might differ among the ultramafic species and help explain the different distributions of the three species in the field and (b) to further investigate the causes of ultramafic exclusion through the responses of the three species of non-ultramafic origin. This experiment was also used to reassess Johnston and Proctor's (1981) work on Festuca rubra.

A third experiment using soil collected at MK1 was set up to compare the growth of the three species pairs' growth in it.

Materials and methods

PLANT SPECIES

Seed material was collected for the three species at MK1, and in addition for F. rubra and C. fontanum in a non-ultramafic acid grassland at about 600 m a.s.l. near the Corrie

of Fee, (NGR 2775, 3725) Caenlochan NNR, Angus, Scotland in 1991. Non-ultramafic material for C. pyrenaica ssp. alpina of non-metalliferous origin was obtained from Dr. A.J.M. Baker's stock collected on Benbulbin (Irish National Grid Reference: G 730, 465), Co. Sligo, Ireland in 1990.

PREPARATION OF SEEDLINGS

Seeds were germinated on wet filter paper in Petri-dishes and seedlings at the cotyledon (Cerastium and Cochlearia), or first leaf stage (Festuca) were carefully put into glass tubes held in holes in the lids of 600-ml beakers. All seedlings were grown initially for a week in a culture solution with 1500 μmol magnesium and no added nickel (the 1.5Mg/0Ni treatment described later).

GROWTH CONDITIONS AND CULTURE SOLUTIONS

Plants in both experiments were grown in a growth room under a photoperiod of 12 h light and 12 h dark with a PAR of 200 $\mu\text{mol m}^{-2} \text{s}^{-1}$ at plant level for 56 d. Temperature was 17 °C during the day and 12 °C during the night. The 600 ml beakers containing the plants were arranged in a completely randomised block design and were rerandomised weekly, when the culture solutions were changed.

Experiment 1

Cochlearia pyrenaica ssp. alpina of ultramafic and of non-ultramafic origin were grown

Compound used	Concentration in culture solution ($\mu\text{mol l}^{-1}$)
$\text{NH}_4\text{H}_2\text{PO}_4$	100
KH_2PO_4	200
$\text{Ca}(\text{NO}_3)_2$	300
NaCl	600
Na_2SO_4	30
NaFeEDTA	10
KFeEDDHA	10
H_3BO_3	4.6
ZnSO_4	0.076
CuSO_4	0.032
$(\text{NH}_4)_6\text{Mo}_7\text{O}_{24}$	0.0074
$\text{Mg}(\text{NO}_3)_2$ †	1500 (1.5Mg); 7500 (7.5Mg)
NaNO_3 †	0 (7.5Mg); 12 000 (1.5Mg)
$\text{Ni}(\text{NO}_3)_2$ †	0 (0Ni); 24.6 (+Ni)
MES buffer*	200 000

Table 4.1 Chemicals and their rate of application in the culture solutions used in Experiments 1 and 2. *MES is 2(N-morpholino)ethanesulphonic acid. † Concentrations of these compounds were experimentally varied and their concentrations in both treatments (with name of the treatment in parentheses) are given in the right-hand column.

in experiment 1. The composition of the culture solutions (Table 4.1) followed that used by Johnston & Proctor (1981) in their experiment No 2 with the modifications that 2(N-morpholino)ethanesulphonic acid (MES) buffer was used to keep solution pH constant after adjusting it to 5.5 by adding NaOH or HCl and KFeEDDHA was used as the iron source according to Chaney (1988) for all species.

Four treatments: (a) 1500 $\mu\text{mol l}^{-1}$ Mg, 0 Ni (1.5Mg/0Ni); 7500 $\mu\text{mol l}^{-1}$ Mg, 0 Ni (7.5Mg/0Ni); 1500 $\mu\text{mol l}^{-1}$ Mg, 24 $\mu\text{mol l}^{-1}$ Ni (1.5Mg/+Ni); and 7500 $\mu\text{mol l}^{-1}$ Mg, 24 $\mu\text{mol l}^{-1}$ Ni (7.5Mg/+Ni) were used. In addition, the Cochlearia's of ultramafic and non-ultramafic origin were grown in a solution containing 10 $\mu\text{mol l}^{-1}$ NaFeEDTA for comparison. Five replicates were used in the solutions with FeEDDHA and three when FeEDTA was used as the iron source.

Experiment 2

Cerastium fontanum ssp. scoticum and C. fontanum ssp. vulgare and Festuca rubra were grown in this experiment using KFeEDDHA only as an iron source. Experiment 2 was similar to Experiment 1 except that, because of the lack of sufficient suitable plant material, the 7.5Mg/+Ni treatment was omitted for all but the Cerastium of non-ultramafic origin. The number of replicates was five in each treatment.

The Cochlearia was not grown in Experiment 2 and the Cochlearia plants grown with KFeEDDHA in Experiment 1 were used for comparing species responses to treatments in the three species pairs.

Experiment 3

Seeds of Cerastium fontanum ssp. scoticum, C. fontanum ssp. vulgare and of Cochlearia pyrenaica ssp. alpina of ultramafic and of non-ultramafic origin of the same stock described earlier were sown in a soil which had been collected at MK1 in 1991 and stored in a growth room for two years. Five seeds per species were sown in thoroughly wetted soil in each of five 14-cm diameter pots in a growth room on 22 April 1993. The growth conditions were identical to those above. The seedlings were inspected for their appearance and their numbers were recorded on two occasions (10 May and 14 June 1993).

ANALYTICAL METHODS

The plant samples were digested in a concentrated sulphuric acid - hydrogen peroxide mix (Allen 1989) in a block digester at 330 °C. Elemental analyses were identical to those described in Chapter II.

STATISTICAL METHODS

Three-way ANOVA's were run for shoot and root dry weight, root weight ratio ($RWR = W_r/W_{r+s}$; W_r is root weight, W_{r+s} is total weight) (Fitter & Hay 1987) and tissue iron and nickel concentration data in Experiment 1. Prior to analyses, data were log-transformed ($\log(x+1)$) to ensure that the factor levels were additive. Multiple comparisons for significant differences were determined by the Tukey test (Zar 1984).

Because of the unequal number of treatments in the species in Experiment 2, one-way ANOVA's were carried out on each species' shoot and root dry weight and RWR.

Results

EXPERIMENT 1

IONIC SPECIATION IN THE CULTURE SOLUTION

Large differences in the ionic composition of the culture solutions with the different chelators were predicted by GEOCHEM (Table 4.2). The GEOCHEM results indicated that the addition of nickel to the solution with NaFeEDTA resulted in all but 4.19% of the iron on the chelator replaced by nickel, which thus had its solution concentration substantially reduced, and iron precipitated as iron phosphate. Also, with no added nickel, copper was bound with EDTA leaving only about 4% as Cu^{2+} ions. When KFeEDDHA was the chelator however there were no appreciable changes in the solution Cu^{2+} and Zn^{2+} on the addition of nickel (of which about 96% remained as Ni^{2+}) and iron remained bound with EDDHA in plant-available form.

COMPARISON OF THE GROWTH OF COCHLEARIA PYRENAICA SSP. ALPINA OF ULTRAMAFIC AND NON-ULTRAMAFIC ORIGIN AT VARYING Mg^{2+} AND Ni^{2+} CONCENTRATIONS IN SOLUTIONS WITH FeEDTA AND FeEDDHA AS THE IRON SOURCE

Shoot dry weight

Treatment	Ion	As free ion %	Bound with EDTA %	As free ion %	Bound with EDDHA %
Control	Fe ³⁺	0	99.6	0	99.9
	Cu ²⁺	3.7	96.2	77.0	19.7
	Zn ²⁺	88.1	13.6	96.3	0
	Ni ²⁺	0	0	0	0
7.5Mg/0Ni	Fe ³⁺	0	99.6	0	99.9
	Cu ²⁺	4.0	95.9	77.0	19.7
	Zn ²⁺	83.1	13.6	96.3	0
	Ni ²⁺	0	0	0	0
1.5Mg/+Ni	Fe ³⁺	0	4.2	0	99.9
	Cu ²⁺	48.1	49.9	77.0	19.6
	Zn ²⁺	95.7	0.6	96.2	0
	Ni ²⁺	59.3	38.9	97.0	0.1
7.5Mg/+Ni	Fe ³⁺	0	4.4	0	99.9
	Cu ²⁺	48.2	49.8	86.8	9.5
	Zn ²⁺	95.8	0.6	96.4	0
	Ni ²⁺	59.5	38.9	97.2	0

Table 4.2 Micronutrient ions in free ionic and chelate-bound form as predicted by Geochem in the culture solutions prepared with NaFeEDTA and KFeEDDHA as iron sources. Values are percentages of total.

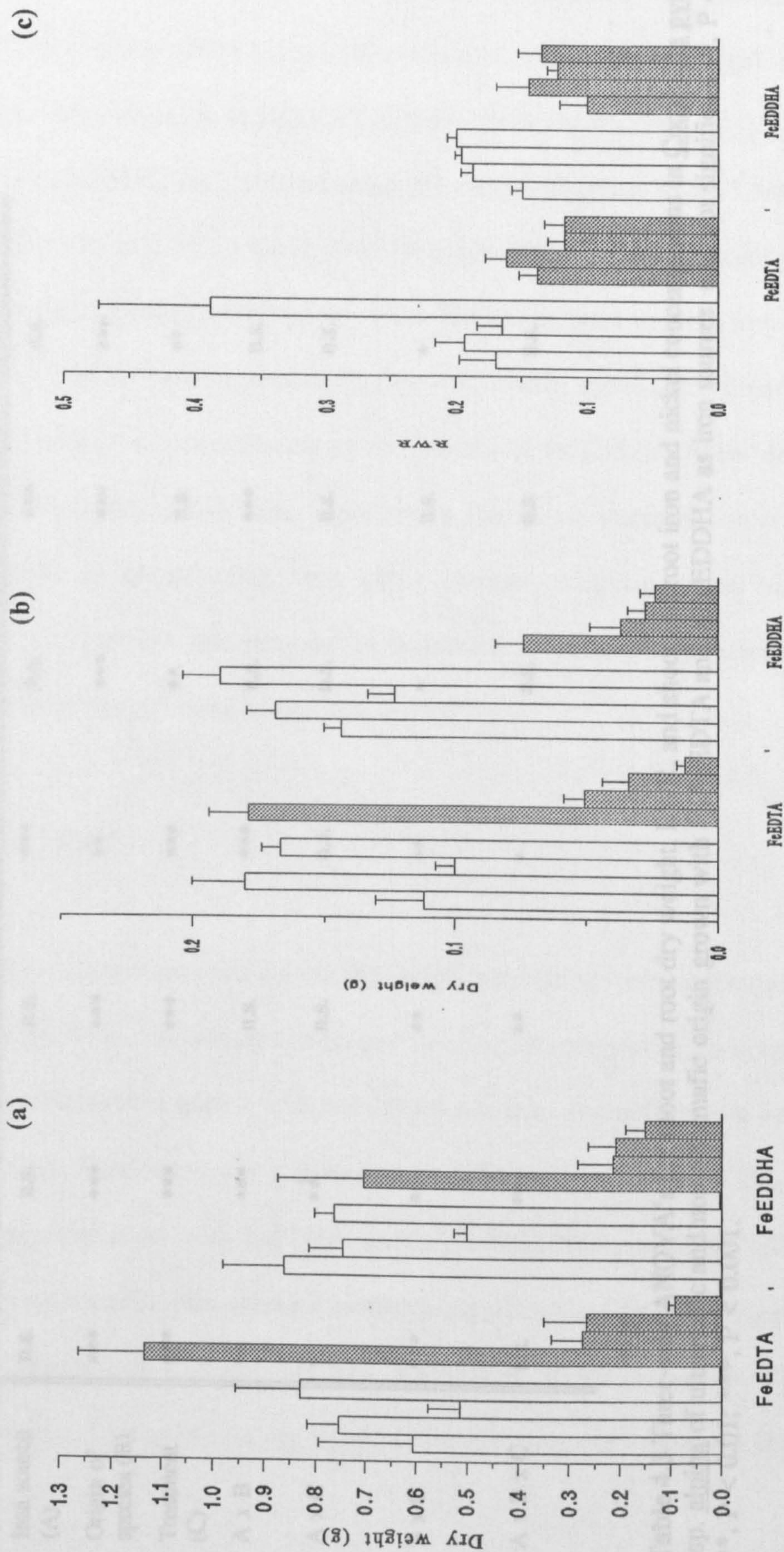


Fig 4.1 Shoot dry weight (a), root dry weight (b) and RWR in *Cochlearia pyrenaica* ssp. *alpina* of ultramafic (unshaded) and non-ultramafic (shaded) origin grown in 1.5Mg/0Ni, 7.5Mg/0Ni, 1.5Mg/+Ni and 7.5Mg/+Ni treatments (from left to right) with FeEDTA and FeEDDHA as iron source. Values are means (n=5) with bars showing s.e.

	Shoot d.w.	Root d.w.	RWR	Shoot iron	Shoot nickel	Root iron	Root nickel
Iron source (A)	n.s.	n.s.	n.s.	***	n.s.	***	n.s.
Origin of species (B)	***	***	***	**	***	***	***
Treatment (C)	***	***	***	***	**	n.s.	**
A x B	**	***	n.s.	***	n.s.	***	n.s.
A x C	*	**	n.s.	n.s.	n.s.	n.s.	n.s.
B x C	***	***	**	**	*	n.s.	*
A x B x C	n.s.	***	**	*	n.s.	n.s.	n.s.

Table 4.3 Three-way ANOVA's for shoot and root dry weight, RWR, and shoot and root iron and nickel concentrations in Cochlearia pyrenaica ssp. alpina of ultramafic and non-ultramafic origin grown with NaFeEDTA and KFeEDDHA as iron sources. n.s., not significant; *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$.

The shoot dry weights of the Cochlearia's in the Mg/Ni treatments with FeEDTA and FeEDDHA are shown in Fig. 4.1a. The iron source alone did not have an overall significant effect on shoot dry weight production, while both the origin of the species and treatment resulted in significantly different shoot growth (Table 4.3). All treatments resulted in a significantly reduced shoot dry weight from that in the 1.5Mg/0Ni in the non-ultramafic species. In the 7.5Mg/0Ni MK1, plants grew significantly better than those of non-ultramafic origin only when FeEDDHA was used as iron source. The addition of nickel with magnesium (7.5Mg/+Ni) caused significantly higher dry weight in plants of both origins when they were grown with FeEDDHA. Shoot dry weights in the non-ultramafic plants were significantly less in all treatments with 7500 μmol magnesium or added nickel, with either chelator, reaching lowest values in the 7.5Mg/+Ni ($p \leq 0.01$). The response to treatments in ultramafic plants did not differ significantly ($p \leq 0.05$) with either iron source.

Root dry weight

The effects of the treatments on root dry weight production were similar to those on the shoots (Fig. 4.1b). Iron source had no overall effect on root growth, however ultramafic plants had better root growth with FeEDDHA and non-ultramafic plants with FeEDTA (Table 4.3). Also, there was a treatment response to iron source. 1.5Mg/0Ni plants produced more roots with FeEDTA than with FeEDDHA, while in 7.5Mg/+Ni the reverse was the case. Iron source also caused significantly different responses between the Cochlearia's of different origin in the various Mg/Ni treatments. There was a pronounced reduction in root dry weight in the non-ultramafic race in 1.5Mg/0Ni with

FeEDDHA ($p=0.001$) while ultramafic plants grew better with FeEDDHA than with FeEDTA. The higher magnesium treatment (7.5Mg/0Ni) caused significantly reduced root production with FeEDTA ($p\leq 0.001$) but not with FeEDDHA ($p=0.055$) in the non-ultramafic Cochlearia. In the ultramafic Cochlearia, magnesium stimulated root growth. Root growth in ultramafic plants was higher than in the non-ultramafic ones in all three treatments with either iron source ($p=0.001$), and in addition ultramafic plants produced significantly higher root mass with FeEDDHA ($p=0.01$) than with FeEDTA. MK1 plants grew significantly better in 1.5Mg/+Ni with FeEDDHA while no difference was found for the non-ultramafic species. With 7.5Mg/+Ni both species grew slightly better in the FeEDDHA-containing solution, however the differences from FeEDTA were not significant.

RWR's were, in the main, determined by the values for roots (Fig. 4.1c). While values were mostly in the range of 0.13-0.20, the RWR was extremely high in the ultramafic plants in the 7.5Mg/+Ni treatment (0.38). Values for the ultramafic plants were significantly higher ($p=0.001$) with both iron chelates (Table 4.3).

Concentrations of iron and nickel in the shoots

Shoot iron concentrations were significantly different in plants grown in the two iron chelators with higher values in the FeEDDHA (Table 4.3). Also, there were significant differences between the two species with higher concentrations in the non-ultramafic plants. Iron concentrations were also significantly different in the different magnesium and nickel treatments independently of chelator type. Races reacted differently to the magnesium and nickel treatments. There were significant differences in the MK1 plants

		ultramafic				non-ultramafic			
	shoot	1.5Mg/0Ni	7.5Mg/0Ni	1.5Mg/+Ni	7.5Mg/+Ni	1.5Mg/0Ni	7.5Mg/0Ni	1.5Mg/+Ni	7.5Mg/+Ni
	nitrogen	-	-	-	-	-	-	-	-
	phosphorus	-	-	-	-	-	-	-	-
	potassium	11400	28200	19100	29800	20600	25200	31700	32500
	calcium	4630	4070	6170	3890	3690	3470	5520	4610
	magnesium	3640	11200	4420	9270	3560	19400	5670	16800
	iron	67	161	108	79	220	638	718	132
	nickel	-	-	154	80	-	-	203	226
	root								
	nitrogen	-	-	-	-	-	-	-	-
	phosphorus	-	-	-	-	-	-	-	-
	potassium	16600	23200	18400	22400	19200	35100	31700	32500
	calcium	4440	3480	4510	3640	5240	1320	4580	3620
	magnesium	4570	14900	4190	14000	4780	11600	6160	7480
	iron	1050	1450	1300	1080	3410	6770	7470	2560
	nickel	-	-	371	260	-	-	342	440

Table 4.4 Elemental concentrations ($\mu\text{g g}^{-1}$ dry weight) of *Cochlearia pyrenaica* ssp. alpina of ultramafic and non-ultramafic origin grown with four magnesium and nickel treatments. Values are means ($n=5$). -, no data.

with either FeEDTA or FeEDDHA. Non-ultramafic plants took up significantly less iron in 7.5Mg/+Ni than in either the 1.5Mg/0Ni, or 7.5Mg/0Ni with FeEDTA. With FeEDDHA a similar pattern was observed, however without significant differences. Differences in mean iron concentrations between the two species were determined by the chelator type used and treatments applied ($p=0.05$). No significant differences were found in the MK1 plants with chelator type, while the non-ultramafic Benbulbin plants contained significantly more iron when grown in the FeEDDHA solutions in each case. Nickel concentrations were not measured in treatments with no nickel addition (Table 4.4). There was no difference with chelators between nickel concentrations in the shoots overall. Significant differences were found between species of different origin with higher nickel concentrations in the non-ultramafic plants ($p=0.001$). Treatments with 7.5Mg/+Ni resulted in lower tissue nickel than 1.5Mg/+Ni ($p=0.01$) with non-ultramafic plants containing higher nickel concentrations in both cases ($p=0.05$ and $p=0.01$).

Concentration of iron and nickel in the roots

There was a significant difference in root iron concentrations with the two types of chelators with FeEDDHA-grown plants having higher iron. Also, plants of non-ultramafic origin contained higher concentrations of iron than those from MK1.

Root nickel concentrations were not different with the two chelators (Table 4.3). The roots of Benbulbin plants contained significantly higher concentrations of nickel than the MK1 plants ($p=0.001$) and in the 1.5Mg/+Ni they contained the highest concentration of nickel ($p=0.05$).

Species	Plant origin							
	ultramafic				non-ultramafic			
	7.5Mg/0Ni	1.5Mg/+Ni	7.5Mg/+Ni	7.5Mg/0Ni	0Mg/+Ni	7.5Mg/+Ni	7.5Mg/0Ni	7.5Mg/+Ni
<u>Cerastium</u> (shoot)	53.6	83.0	n. a.	83.0	74.6	80.5		
<u>Cochlearia</u> (shoot)	86.5	58.2 *	88.5	30.4 **	29.4 **	21.3 **		
<u>Festuca</u> (shoot)	103	95.8	n. a.	14.8 ***	13.1 ***	n. a.		
<u>Cerastium</u> (root)	49.8 *	95.8	n. a.	60.0 ***	93.3	65.7 ***		
<u>Cochlearia</u> (root)	118	85.7 *(1Mg/0Ni)	132 *(0Mg/1Ni)	50.3	37.6 *	32.4 *		
<u>Festuca</u> (root)	86.9	99.7	n. a.	6.6 ***	9.9 ***	n. a.		
<u>Cerastium</u> Wr/Wr+s	93.3	113	n. a.	73.8	122 *** (1Mg/0Ni)	82.7 *(0Mg/1Ni)		
<u>Cochlearia</u> Wr/Wr+s	126	131	134	145	123	135		
<u>Festuca</u> Wr/Wr+s	89.4	118	n. a.	39.3 **	84.0	n. a.		

Table 4.5 Relative shoot and root dry weights and root weight ratios (Wr/Wr+s) in Cerastium fontanum, Cochlearia pyrenaica and Festuca rubra of ultramafic and non-ultramafic origin grown in four Mg/Ni treatments. Values given are treatment means expressed as percentages of the ultramafic and non-ultramafic plant origin grown with 1.5Mg/0Ni. Significant differences shown are those from controls (*, p=0.05; **, p=0.01; ***, p=0.001) unless otherwise indicated in superscripts; n. a., data not available.

EXPERIMENT 2

COMPARISON OF GROWTH AND CHEMICAL COMPOSITION OF CERASTIUM FONTANUM, COCHLEARIA PYRENAICA AND FESTUCA RUBRA IN VARIOUS Mg/Ni TREATMENTS

Dry weights

Table 4.5 summarises the relative shoot and root dry weight production and RWR's in the different treatments for Cerastium fontanum, Cochlearia pyrenaica, and Festuca rubra. The different Mg/Ni treatments caused no significant changes in shoot dry matter production in Cerastium of either origin but 7.5Mg/0Ni in the ultramafic and 7.5Mg/0Ni and 7.5Mg/+Ni in the non-ultramafic plants brought about a 35-50% reduction in root mass ($p \leq 0.05$; $p \leq 0.001$, $p \leq 0.001$).

The only significant response to treatment in the MK1 Cochlearia was in the 1.5Mg/+Ni, where shoot dry weight was reduced by about 40% relative to the 1.5Mg/0Ni ($p \leq 0.05$). There was also a slight reduction in root mass in the 1.5Mg/+Ni, however this was not statistically different from the 1.5Mg/0Ni. The addition of magnesium resulted in increased root masses, which together with the reduction in the 1.5Mg/+Ni led to significant differences between treatments with extra magnesium (7.5Mg/0Ni and 7.5Mg/+Ni) and added nickel without extra magnesium (1.5Mg/+Ni). The Cochlearia of non-ultramafic origin showed dramatic responses to the addition of 7500 $\mu\text{mol l}^{-1}$ magnesium or nickel and produced 70-80% less shoot dry weight. The reduction in root mass was also significant in all but the 7.5Mg/0Ni treatments.

There were no effects of magnesium or nickel addition on the shoot and root dry weight in the Festuca of ultramafic origin. Growth in the plants of non-ultramafic origin on the

	ultramafic				non-ultramafic			
	1.5Mg /ONi	7.5Mg/ONi	1.5Mg/+Ni	7.5Mg/+Ni	1.5Mg /ONi	7.5Mg/ONi	1.5Mg/+Ni	7.5Mg/+Ni
shoot	25700	31700	27100	-	28800	26200	28200	24500
nitrogen	6800	7370	7420	-	3590	3270	4650	3340
phosphorus	16200	28700	18700	-	14500	17300	14900	16700
calcium	1760	1660	1950	-	1160	874	2060	936
magnesium	3990	13000	6170	-	4020	8520	5270	8610
iron	25	274	45	-	30	22	18	10
nickel	-	-	84	-	-	-	100	54
root								
nitrogen	53500	46500	48600	-	48300	44500	45600	41900
phosphorus	14400	13200	13500	-	10800	5780	11900	5980
potassium	16600	29600	15600	-	12300	9800	11300	8210
calcium	4350	5640	4420	-	4850	3470	3790	2190
magnesium	5550	6590	6170	-	5140	7120	5090	6560
iron	376	478	1260	-	861	820	1800	825
nickel	-	-	1040	-	-	-	1350	528

Table 4.6 Elemental concentrations ($\mu\text{g g}^{-1}$ dry weight) of *Cerastium fontanum* of ultramafic and non-ultramafic origin grown in four magnesium and nickel treatments. Values are means (n=5). -, no data.

		ultramafic				non-ultramafic			
		1.5Mg/0Ni	7.5Mg/0Ni	1.5Mg/+Ni	7.5Mg/+Ni	1.5Mg/0Ni	7.5Mg/0Ni	1.5Mg/+Ni	7.5Mg/+Ni
shoot									
nitrogen		44600	46600	44500	-	33800	41000	49100	-
phosphorus		6940	7790	7400	-	4140	5410	6520	-
potassium		20200	21500	21700	-	13400	19500	26400	-
calcium		1880	2590	2700	-	2310	2190	4840	-
magnesium		2420	9540	3720	-	3530	17800	4690	-
iron		54	86	65	-	28	70	68	-
nickel		-	-	141	-	-	-	190	-
root									
nitrogen		37000	-	36700	-	-	41600	34800	-
phosphorus		6620	7690	7110	-	5780	4710	6120	-
potassium		11800	9090	-	-	17000	19100	17700	-
calcium		1220	7690	1050	-	5781	4469	1785	-
magnesium		2320	2650	1750	-	2100	8370	1390	-
iron		127	202	161	-	148	713	532	-
nickel		-	-	2440	-	-	-	2030	-

Table 4.7 Elemental concentrations ($\mu\text{g g}^{-1}$ dry weight) of *Festuca rubra* of ultramafic and non-ultramafic origin grown with four magnesium and nickel treatments. Values are means ($n=5$). -, no data.

other hand was very much depressed in the 1.5Mg/+Ni and 7.5Mg/0Ni treatments. They only produced 15% (7.5Mg/0Ni) and 13% (1.5Mg/+Ni) shoot dry matter and 7% and 10% root mass when compared with the plants in the 1.5Mg/0Ni solution.

Root weight ratios of the ultramafic plants did not differ from the 1.5Mg/0Ni in either species. Non-ultramafic species showed differential responses. RWR was significantly higher in the 1.5Mg/+Ni than in 7.5Mg/0Ni and 7.5Mg/+Ni in Cerastium. Although there was an increase of 22-44% in Cochlearia it was not significant ($p \leq 0.05$). In Festuca added magnesium (7.5Mg/0Ni) significantly reduced the RWR.

Chemical composition

Tables 4.4, 4.6 and 4.7 show the elemental composition of the three species pairs in the different Mg/Ni treatments.

EXPERIMENT 3

100% of the Cerastium fontanum ssp. scoticum emerged and 96% survived by June and the plants were vigorous and green. Conversely, only 84% emerged in the C. fontanum ssp. vulgare and the surviving plants (25% of the seedlings died) were minute, yellow and apparently dying.

There was no reduction in the number of surviving plants in the ultramafic Cochlearia: 72% emerged by May and was increased to 76% by June. Only 48% of the non-ultramafic seeds emerged in May and two-thirds of them died by June and the remaining one-third were also dying.

Discussion

EXPERIMENT 1

In the original hypothesis it was assumed that the iron sources would not cause differential growth responses in the Cochlearia's of different origin. This was not the case however. There was a depression of growth in the non-ultramafic plants in the 1.5Mg/0Ni solution with KFeEDDHA, which was significant in the roots. This may suggest that plants may have taken up some chelator which caused restricted growth. There is no evidence for this however. Fe-chelates are not taken up intact; Fe^{3+} is reduced to Fe^{2+} and split off the chelator before uptake. The amount of chelators taken up is thought to be very small with no effects on plant growth (Weaver et al. 1984, Halvorson & Lindsay 1977). It is also possible that the depression in growth is connected with the two to five-fold higher iron concentrations in the roots and shoots of the Benbulbin plants in the FeEDDHA-containing solution. It also should be born in mind that the number of replicates was only three and inferences should be made with caution.

Of the nickel-treated MK1 plants, 7.5Mg/+Ni with EDDHA caused significantly higher shoot production and root growth was also increased when compared with EDTA. Shoot iron was higher with FeEDDHA but not significantly so. Nickel concentrations in both were not significantly different with the two iron sources. The improved growth may have possibly been caused by the higher internal Fe/Ni quotient, a factor which has been suggested as of importance in tolerance to nickel (Mizuno & Nosaka 1992). However, it cannot be ruled out that the much increased availability of Cu^{2+} in the

solution with EDDHA (Table 4.2) was also a contributing factor. The concentration of micronutrients (unspecified) in culture solutions was found to have important effects in the simulated MK1 soil solution work of Johnston & Proctor (1981). Non-ultramafic Cochlearia also benefited, however not significantly, from the use of FeEDDHA which may be explained by its higher root iron, which may have afforded some ameliorative effect on the toxicity of free Ni²⁺. Also the above-mentioned increased availability of Cu²⁺ is another possible explanation.

It appears that although there was some different responses to the chelates it did not fundamentally affect the conclusions from the present experiment. Species effects were much more significant. The species x iron chelate interaction observed in the 1.5Mg/0Ni however suggests control species for comparison in future ecotoxicological work must be carefully selected. It is undesirable to have species pairs (or races) for comparative ecological and ecophysiological work which respond differently to factors which are not of prime interest since they may also confound inferences from true treatment effects. It seems that not only is the choice of iron source important for hydroponic systems but attention should be paid to the species x chelator interactions to best reproduce field conditions for iron availability.

Based on the findings of Experiment 1 and those concerning the growth of Festuca rubra with FeEDTA (Johnston & Proctor 1981) and with FeEDDHA (Experiment 2, present study) it seems likely that Johnston & Proctor's (1981) results were probably affected by the use of FeEDTA, but their overall conclusions are not invalidated.

EXPERIMENT 2

Comparison of the growth of Cerastium fontanum, Cochlearia pyrenaica and Festuca rubra of ultramafic origin in different Mg/Ni treatments

The three species of ultramafic origin responded differently to the addition of magnesium and nickel to the culture solution. Cerastium fontanum ssp. scoticum grew less well in the presence of high magnesium; the addition of nickel did not cause a significant reduction in shoot production; and root dry weight was not affected at all by added nickel. It may be that the significant reduction in root growth was caused by magnesium toxicity as was suggested by Proctor & McGowan (1976) for oats. Kinzel (1982) suggested that there may exist a detoxifying mechanism in ultramafic plants, especially in the Caryophyllaceae, based on the formation of magnesium-oxalate crystals in the vacuoles analogous to the formation of calcium-oxalate in calciphobic plants. If this mechanism existed in C. fontanum ssp. scoticum then one might expect the species to be indifferent to magnesium. However, histological work on Cerastium plants collected at MK1 failed to show magnesium-oxalate crystals in the leaf tissue. The shoot calcium concentration does not suggest that translocation of this element to the shoots was inhibited, though a small, but insignificant reduction occurred in the shoots in the 7.5Mg treatments.

The concentrations of magnesium and nickel used in this experiment were based on the soil solution extracts of Johnston & Proctor (1981) and reflect the 'availability' of these elements in the prevailing wet soil conditions. It is tentatively suggested therefore that soil magnesium may influence the occurrence and reproductive ability of C. fontanum

ssp. scoticum at MK1 with nickel probably having no effect.

The response to nickel and magnesium in Cochlearia contrasted with that in Cerastium. Magnesium did not reduce shoot growth significantly and root growth was even slightly stimulated. This latter may be connected with the generally two-fold higher calcium concentrations in the shoots and in the roots in Cochlearia than in Cerastium. The addition of nickel, on the other hand, significantly reduced shoot growth and also but not significantly root growth. It is noticeable that nickel concentrations in Cochlearia were about two-fold higher than in Cerastium both in the shoots and roots. Although it is not known in what form nickel was present, this two-fold higher concentration may explain the greater growth reduction in Cochlearia. It is interesting that reduction of root growth was only slight and not significant since this is a characteristic nickel toxicity symptom (Woolhouse 1983) and has been reported recently in Zea mays by Robertson (1985). It has been long suggested that in certain plants magnesium ameliorated nickel toxicity (Crooke 1956, Gabbrielli & Pandolffini 1984, Proctor & McGowan 1976). The response of the MK1 Cochlearia in this experiment supports these earlier findings.

It appears that for Cochlearia, the soil nickel concentration and the Ni/Mg quotient may be of importance for growth on the ultramafic site.

The growth of ultramafic Festuca rubra was not affected by nickel or magnesium. Festuca had the lowest concentrations of shoot magnesium in the 1.5Mg/0Ni although its uptake of the element in the high magnesium solution was similar to the other species. There was no increased calcium uptake on the addition of nickel although this occurred in all the non-ultramafic races and in the MK1 Cochlearia and is generally considered as a response to metal toxicity stress to maintain membrane integrity. Also, in contrast to the other two species, potassium concentrations remained unchanged in

		ultramafic K/(K+Na)		non-ultramafic K/(K+Na)	
		630 $\mu\text{mol l}^{-1}$ solution Na	12000 $\mu\text{mol l}^{-1}$ solution Na	630 $\mu\text{mol l}^{-1}$ solution Na	12000 $\mu\text{mol l}^{-1}$ solution Na
<u>Cochlearia</u>	1.5Mg/0Ni	-	0.30	-	0.43
	7.5Mg/0Ni	0.82	-	0.80	-
	1.5Mg/+Ni	-	0.37	-	0.49
	7.5Mg/+Ni	0.81	-	0.81	-
<u>Festuca</u>	1.5Mg/0Ni	-	0.54	-	0.70
	7.5Mg/0Ni	0.97	-	0.97	-
	1.5Mg/+Ni	-	0.62	-	0.70
	7.5Mg/+Ni	n.a.	-	n.a	-

Table 4.8 The values of potassium/(potassium+sodium) quotients (K/(K+Na)) in the shoots of Cochlearia pyrenaica ssp. alpina and Festuca rubra of ultramafic and non-ultramafic origin grown in various Mg/Ni treatments. -, not applicable; n.a., data not available.

all treatments in Festuca. This apparent tolerance to both toxicities adds to the cases of the well documented ability of Poaceae to evolve and maintain tolerance to metal toxicities through an array of biochemical and physiological means (Woolhouse 1983). An explanation for the higher shoot potassium concentrations in each species in treatments where they suffered the highest depression in growth may be part of the 'Viets effect' (Viets 1944), and result from an increased calcium uptake to maintain membrane integrity in the presence of nickel. However, potassium uptake was also influenced by solution sodium concentration through the partial replacement of K^+ by Na^+ in the treatments with $1500 \mu\text{mol l}^{-1}$ magnesium and $12000 \mu\text{mol l}^{-1}$ sodium (Table 4.8). Johnston & Proctor (1981) argued that the similarly differing sodium concentrations in their experiments were unlikely to have had a major effect on the growth of Festuca rubra. Sodium can, however, stimulate growth in species where there is no exclusion mechanism in operation to prevent Na^+ translocation to the shoots (Marschner 1986) through cell expansion causing higher leaf area when a high proportion of shoot K^+ is replaced by Na^+ (Milford et al. 1977). The addition of the $12000 \mu\text{mol l}^{-1}$ sodium caused four- to five-fold increases in the shoot sodium reducing the $K/(K+Na)$ quotient from 0.80 to 0.30-0.37 in the ultramafic and from 0.80 to 0.43-0.49 in the Benbulbin Cochlearia plants. In Festuca, the $K/(K+Na)$ quotient was 0.97 in the 7.5Mg/0Ni in plants of both origin and was reduced to 0.54-0.62 in the ultramafic and 0.70 in the non-ultramafic plants with $12000 \mu\text{mol l}^{-1}$ sodium. The difference in the uptake of sodium suggested different strategies in the two species. Cochlearia apparently reacted by replacing more K^+ by Na^+ as well as increasing the total of two ions. This 'includer' strategy (Marschner 1986) could probably be related to the taxonomy of the species: most Cochlearia species are maritime. In contrast, Festuca hardly took up any

sodium when grown at low solution sodium concentrations and even at the high concentrations the uptake was more in favour of potassium, more so in the non-ultramafic plants. Nickel caused an increase in the total tissue concentrations of the two ions.

The growth responses to added nickel or magnesium in Festuca suggest that the species' distribution within the Meikle Kilrannoch ultramafic site is not controlled by variations in these soil chemical factors.

Two water culture experiments with Lychnis alpina failed because of the difficulty of growing this species in hydroponics. About 5% of the plants survived in each of the treatments. Those in 1.5Mg/0Ni, 7.5Mg/0Ni, and 1.5Mg/+Ni were green while those in the 7.5Mg/+Ni showed red coloration and were similar in appearance to plants in the field.

The responses by the species of non-ultramafic origin

Cerastium of non-ultramafic origin behaved similarly to the MK1 C. fontanum ssp. scoticum in that root growth was significantly less with added magnesium (7.5Mg/0Ni and 7.5Mg/+Ni) with shoot growth not affected. The lower production of roots and the general appearance of the roots - they showed profuse branching with very short lateral roots - suggests that the vitality of the root tip meristems may have been lessened. It is not established whether it was caused by direct toxicity by magnesium or by a severe reduction of available calcium for meristematic activity, or both. Restricted calcium uptake cannot be ruled out as in the 7.5Mg/0Ni, shoot calcium was significantly lower than in the 1.5Mg/0Ni ($p=0.05$) and the 1.5Mg/+Ni plants contained higher calcium than

in any of the other treatments.

The response of Cerastium was unusual as previous work usually showed that non-ultramafic plants were capable of only poor growth on ultramafic media (Main 1981, Proctor 1971, Walker 1954). It should be noted, however, that Cerastium grown in the growth room on soil collected at the site could only reach the cotyledon with-first leaf-pair stage in contrast with the ultramafic ssp. scoticum the growth of which showed no reduction.

Both Cochlearia and Festuca grew badly in the 7.5Mg/0Ni, 1.5Mg/+Ni and 7.5Mg/+Ni solutions. Although shoot growth was significantly depressed in Cochlearia in all treatments compared with the 1.5Mg/0Ni, root growth in the 7.5Mg/0Ni was less affected than in the other two treatments where they were significantly different from the 1.5Mg/0Ni. The shoot concentration of nickel was not significantly different in the non-ultramafic and ultramafic plants which suggests that ultramafic plants grew better in the presence of nickel because they were able to inactivate it by one of the several means suggested (e.g. Brooks 1987, Triffin 1971).

It was not possible to determine whether nickel or magnesium was more detrimental to the growth of Festuca in contrast to Johnston & Proctor (1981) who found the greater growth reductions were associated with magnesium.

Caution is required when extrapolating to the field from laboratory experiments (Rorison 1969). Even the laboratory pot trial in Experiment 3 showed for both the non-ultramafic species that though seeds germinated the seedlings soon became chlorotic and were unable to establish. Clearly there is a difference between conditions prevailing in the soil and the culture solutions used. The difference may simply lie in the readier availability of nutrients for uptake in the nutrient solution. It is also possible that other,

ions which were not investigated may be of importance at the establishment stage for plants of non-ultramafic origin.

It seems that different factors govern ultramafic exclusion in the non-ultramafic races of the three species investigated. Cochlearia and Festuca are likely to be prevented from growing on ultramafics by the lack of tolerance to either nickel or high magnesium. The Cerastium data on the other hand are more difficult to interpret. Both races show a depression of growth in response to high magnesium concentrations in the culture solution they grow fairly well in it nonetheless. The failure of the non-ultramafic race in the soil indicates that some unknown factor is of importance there.

Chapter V

The effects of different concentrations of available iron and nickel on the growth of Cochlearia pyrenaica ssp. alpina of ultramafic and non-ultramafic origin and of C. officinalis

Introduction

Nickel toxicity has been proposed as one of the causes for specialised vegetation on ultramafic soils (for a recent review see Proctor & Baker 1994) and the importance of sufficient internal iron concentrations in countering the deleterious effects of nickel have been proposed for many living organisms (e.g. Tandor & Mather 1986, Mizuno & Nosaka 1992). Earlier work had shown the ameliorative effect of iron on nickel toxicity in oats grown in culture solutions (Crooke et al. 1954). Japanese workers have found that plants with internal Fe/Ni quotients higher than about 5 have suffered no toxicity or at least less than those in which this quotient fell below 5 (Mizuno & Nosaka 1992). It has also been demonstrated experimentally that iron plaque formed on the root surface of wetland plants has reduced growth loss caused by the addition of nickel to the growth medium (Greipson & Crowder 1991).

Investigations on the physiological effects of nickel in plants have included measurements of net photosynthesis, chlorophyll fluorescence, respiration, stomatal regulation of transpiration and overall water relations. Net photosynthesis and

transpiration responses to Ni^{2+} in whole maize and sunflower plants have been reported by Carlson and co-workers (Carlson *et al.* 1975). The inhibitory effect of nickel on photosynthetic electron transport has been shown in Nostoc muscorum, a blue-green alga (Singh *et al.* 1991) and in the phanerogam Ocimum basilicum (Lamiaceae) (Veeranjaneyulu & Das 1982).

Vergnano & Hunter (1953) have suggested that nickel produced visual symptoms and anatomical changes in oats similar to those induced by iron deficiency. In an experiment (a precursor to that described in Chapter IV) on Cochlearia plants it was found that Ni^{2+} treatment caused mild chlorosis in mature leaves and further leaf development was inhibited in plants from a non-metalliferous site. No visual symptoms were observed in plants of ultramafic origin though their dry matter production was decreased in the nickel treatments. It was thought that chlorosis in non-adapted plants was caused essentially by iron deficiency probably brought about by the replacement of iron by nickel which presumably did not functionally substitute for it. Also, nickel uptake was higher in non-adapted plants than in the ultramafic ones probably owing to a breakdown of selectivity caused by nickel toxicity as reported for maize by Robertson (1985).

The main objective of this study was to test the hypothesis that Cochlearia pyrenaica ssp. alpina of ultramafic origin is more nickel tolerant because it can keep its photosynthetic system (and probably also other Fe-containing enzymes involved in electron transport processes such as cytochromes in the photosynthetic electron transport system, catalase, peroxidase, ferredoxin) less affected by nickel, while in the non-ultramafic congeneric species, reduction in growth is caused by lower rates of net photosynthesis owing to the inhibitory effect of an Fe/Ni imbalance on the photosynthetic electron transport system.

It was also the intention of this study to obtain information about the sites of action of nickel.

Materials and methods

PLANT SPECIES

Cochlearia pyrenaica ssp. alpina of non-metalliferous origin, C. pyrenaica ssp. alpina of ultramafic origin and C. officinalis of sea-cliff origin were grown in culture solution. The seeds of C. pyrenaica ssp. alpina were collected at the ultramafic site MK1 (see Chapter IV) in 1991 and were stored at room temperature until the experiment. Seeds of C. officinalis collected at Polcurran Cliffs, Cornwall, England and C. pyrenaica ssp. pyrenaica from Benbulbin, Co. Sligo, Ireland (Irish NGR: G 730, 465) were obtained from Dr. A.J.M. Baker's collection and prior to receiving them they were kept in deep freeze. Germination tests showed about 95% germination for C. pyrenaica ssp. alpina from MK1 and C. officinalis while it was about 75% for C. pyrenaica ssp. alpina from Benbulbin.

For the experiment the seeds were put to germinate on damp filter paper in Petri-dishes at room temperature for one week. Emerging seedlings were carefully threaded through small glass tubes by squirting deionised water from a wash bottle with a fine nozzle. The tubes were to hold plants in place in the cover lids. The seedlings fitted in the tubes were then transferred to a 'nursery' solution, which was identical with the control (1Fe/0Ni) treatment in the experiment, and were kept there until the first foliage leaves appeared one week later.

CULTURE SOLUTION

The culture solution was based on that used in Experiment 2 in Johnston & Proctor (1981) with the exception of the iron source. Following Chaney's cautioning about the possible confounding effects of the use of NaFeEDTA in micronutrient experiments (R.L. Chaney personal communication, Chaney *et al.* 1989) EDDHA was used as an iron chelating agent (*viz.* Chapter IV).

The following combinations of iron and nickel concentrations were used in the experiment: (1) no iron or nickel (0Fe/0Ni); (2) 10 $\mu\text{mol l}^{-1}$ iron, no added nickel (1Fe/0Ni); (3) 10 $\mu\text{mol l}^{-1}$ iron, 24.6 $\mu\text{mol l}^{-1}$ nickel (1Fe/1Ni); (4) 100 $\mu\text{mol l}^{-1}$ iron, 24.6 $\mu\text{mol l}^{-1}$ nickel (2Fe/1Ni); (5) 10 $\mu\text{mol l}^{-1}$ iron, 123.0 $\mu\text{mol l}^{-1}$ nickel (1Fe/2Ni); and (6) 100 $\mu\text{mol l}^{-1}$ iron, 123.0 $\mu\text{mol l}^{-1}$ nickel (2Fe/2Ni).

Stock solutions of 100-strength of $\text{NH}_4\text{H}_2\text{PO}_4$, KH_2PO_4 , $\text{Ca}(\text{NO}_3)_2$, Na_2SO_4 , $\text{MnSO}_4 \cdot 4\text{H}_2\text{O}$, HBO_3 and 1000-strength of $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$, $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$, $(\text{NH}_4)_6\text{Mo}_7\text{O}_{24} \cdot 6\text{H}_2\text{O}$ and $\text{Mg}(\text{NO}_3)_2$ were made up. These were diluted appropriately and the culture solutions were adjusted to pH 6.0 (with the addition of NH_4OH) and changed weekly. MES buffer of the same concentration as in Chapter IV was used to keep solution pH constant over the period between changes.

Plants were grown in a growth room under a photoperiod of 16 h light and 8 h dark with a PAR of 200 $\mu\text{mol m}^{-2} \text{s}^{-1}$ at plant level. The temperature was 20 °C during the day and 15 °C during the night.

GROWTH ANALYSIS

To follow the dynamics of the growth of plants in the different treatments, leaf area expansion was recorded at weekly intervals for 28 d beginning one day after setting up the experiment. Two measurements were made on each leaf for each plant: maximum width and length of lamina. To determine the relationship between the measured values and actual leaf area, a number leaves were traced onto a clean sheet of paper, cut out, weighed on an analytical balance and the areas were calculated from the weighed values. The obtained leaf area values were plotted against the products of the maximum widths and lengths of the same leaves. A functional relationship was established by curve fitting using Cricket Graph software package. Absolute and relative growth rates (G , $(\text{cm}^2 \text{7d}^{-1})$ and R , $(\text{cm}^2 \text{cm}^{-2} \text{7d}^{-1})$) were calculated from the derived untransformed leaf area values.

Shoot and root dry weights were also determined for plants in each of the six treatments originally set up. Plants were transferred to beakers filled with deionised water for 1 min then rinsed in running deionised water in 1 min. Roots and shoots were separated and put to dry in a drying cabinet at 70°C for 48 h.

ELEMENTAL ANALYSIS OF LEAVES

Air dried leaf blades were digested in a sulphuric acid-hydrogen peroxide mixture (Allen 1989) using a block digester at 330°C . The digests were filtered (Whatman No 44) and made up to 100 ml. Analyses were identical with those described in Chapter IV.

MEASUREMENTS OF OXYGEN EVOLUTION

Only plants from the 1Fe/0Ni, 1Fe/1Ni and 2Fe/1Ni treatments were investigated as the other treatments (1Fe/2Ni and 2Fe/2Ni) killed the plants, or were discontinued (0Fe/0Ni).

A Hansatech MKI LD-2 oxygen electrode system (Hansatech, King's Lynn, U.K.) was used to measure oxygen evolution, or depletion. Oxygen uptake in the dark and oxygen evolution on illumination were measured in detached laminae of the three species at saturating CO₂ concentration generated by using a Na₂CO₃/NaHCO₃ buffer. Leaf temperature was maintained at 20 °C by circulating water from a constant temperature bath through the top section of the chamber lid which was in contact with the leaves. A LH36UB ultra-bright red LED array light source (Hansatech, King's Lynn, U.K.) was used to illuminate the leaves in the range of photon flux densities (PFD-s) of 0-1164 μmol m⁻² s⁻¹ to produce light saturated photosynthesis. An IBM compatible computer with the computer programme 'LeafDisc' was used to control the illumination and calculate oxygen evolution.

Oxygen quantum yield

The quantum yield of photosynthesis (calculated as the molar amount of oxygen evolved per moles of photons incident on the leaves) was determined for each treatment in the three species. Identical photon yields would indicate no difference in photosynthetic efficiency, while a decrease in yield would suggest that the photosynthetic system suffered some stress inhibition. The initial portion of the O₂/PFD

curves, where the rate of photosynthesis is directly proportional to PFD, above the light compensation point was used to calculate quantum yield.

CHLOROPHYLL CONCENTRATION

Chlorophyll concentration was determined in the leaves used for the polarographic determination of oxygen evolution. Prior to extraction the leaves were wrapped up in wet tissue paper and tinfoil and kept at -80 °C. Extraction was made in batches of five to ensure rapid processing to avoid damage to the chlorophylls. The leaves were homogenised after thawing using a pestle and mortar and extracted in 80% acetone at 0 °C. The samples were made up to 14 ml by adding more 80% acetone and kept on ice in the dark until they were centrifuged at 4000 r.p.m. for 5 min. The supernatant was used to measure absorbances on a spectrophotometer at 663 and 646 nm wavelengths for chlorophyll a and chlorophyll b. The amount of chlorophylls per unit leaf area was calculated according to Lichtenthaler & Willburn (1983): total chlorophyll = $(8.02 \times OD_{663}) + (20.20 \times OD_{646})$.

CHLOROPHYLL FLUORESCENCE

Chlorophyll fluorescence measurements were made to detect possible inhibitory effects of nickel on the photosynthetic system (PSII) in nickel-treated plants. The fast phase (initial first second of illumination) of chlorophyll fluorescence is related to primary processes in PSII. There are two main stages during the fast phase: initial fluorescence (F_0) before photochemical processes occur, indicating open reaction centres; and

maximum fluorescence (F_m), indicating the total closure of reaction centres. F_0 and F_m were measured by using a PAM Fluorometer 101 (Walz, Effeltrich, Germany). Plants were dark adapted for 1 min before each measurement; then very low light was applied to yield F_0 without inducing photosynthesis; then a saturating flash was applied from an actinic source to close all reaction centres and yield F_m . Measurements were done on one young and one mature leaf for each individual. Variable fluorescence/maximum fluorescence (F_v/F_m) quotients were calculated from $(F_m - F_0)/F_m$ to establish any damage to primary photochemistry.

STATISTICAL ANALYSIS

All data were subjected to logarithmic transformation to meet the criterion of additivity of factor effects for analysis of variance. For R and G the formula $y = \log(x+1)$ was used while the rest of the values were $y = \log(x)$ transformed.

Two-way analyses of variance were carried out to test for treatment and species effects on foliage area, G and R, leaf chlorophyll a+b concentration, and for data from the elemental analysis and the polarographic measurements of photosynthesis.

Results

FOLIAGE AREA, R AND G

There were no differences between leaf area in the different species or treatments at the beginning of the experiment (Table 5.1). Overall foliage area was highest (significantly

	L1	L2	L3	L4	L5	R1	R2	R3	R4	G1	G2	G3	G4
Species (A)	n.s.	***	***	*	*	***	n.s.	n.s.	n.s.	***	**	n.s.	n.s.
Treatment (B)	n.s.	n.s.	**	**	**	n.s.	***	**	n.s.	n.s.	***	**	**
A*B	n.s.	**	*	*	*	**	n.s.	*	n.s.	**	n.s.	**	*

Table 5.1 Two-way ANOVA's for leaf area (L1-L5), relative growth rate (R1-R4) and absolute growth rate (G1-G4) in Cochlearia pyrenaica ssp. alpina of ultramafic and non-ultramafic origin and C. officinalis of maritime origin in various Fe/Ni treatments (n=3). Leaf area was calculated at weekly intervals.

higher than in the non-ultramafic conspecific at 7 d, 14 d and 21 d) in the ultramafic Cochlearia pyrenaica ssp. alpina. C. officinalis also had a significantly larger leaf area at 7 d and 14 d than the non-ultramafic C. pyrenaica. Treatment effects became apparent after 14 d with C. officinalis growing significantly better in the 1Fe/0Ni than in either the 1Fe/1Ni or 2Fe/1Ni, and the non-ultramafic C. pyrenaica produced significantly higher leaf area in the 1Fe/0Ni than those in the 1Fe/1Ni. The differences were maintained throughout the duration of the experiment. No differences with treatment in leaf area were found in the ultramafic species.

The results of the two-way ANOVA's for R at the four times of its calculation in the three species grown in the 1Fe/0Ni, 1Fe/1Ni, and 2Fe/1Ni treatments are shown in Table 5.1. The significant species-effect apparent after 7 d growth arising from better initial growth by Cochlearia pyrenaica ssp. alpina (u. m.) and C. officinalis almost disappeared after 14 d and was gone by 21 d. Differences in R between treatments, initially owing to the significantly higher R for C. officinalis in the 1Fe/0Ni at 7 d, then to the differences between the 1Fe/0Ni and all the treatments with nickel in C. officinalis and Cochlearia pyrenaica ssp. alpina (non-u. m.), were sustained for 21 d. These differences were eliminated after 28 d.

There were significant differences between the absolute growth rates (G_w) in the three species in the early period of growth (7 d and 14 d) with Cochlearia pyrenaica ssp. alpina (non-u. m.) showing the least G_w . Treatment effects were significant throughout because 1Fe/0Ni plants grew better than the 1Fe/1Ni and 2Fe/1Ni in the non-ultramafic species.

DRY WEIGHT

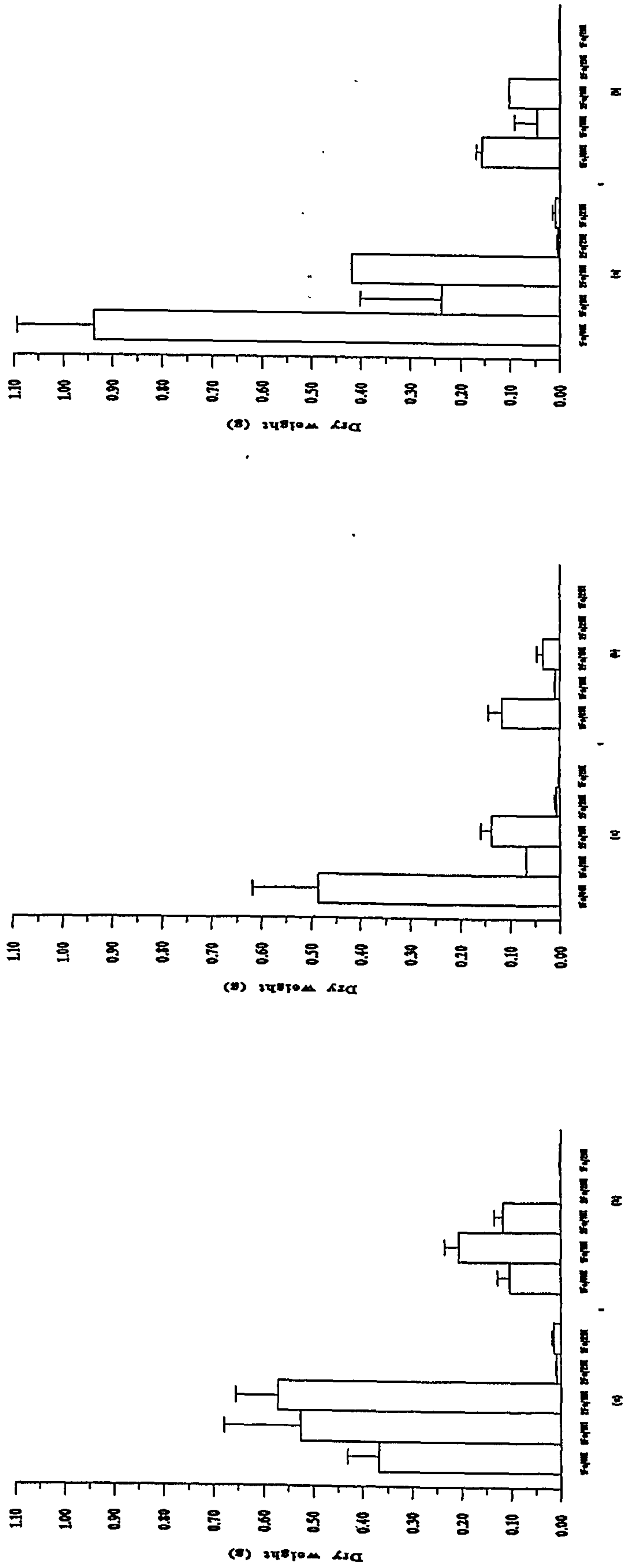


Fig 5.1 Dry weight production in the shoots (a) and roots (b) in *Cochlearia pyrenaica* ssp. *alpina* (u. m.), *C. pyrenaica* ssp. *alpina* (non-u. m.) and *C. officinalis* grown in 1Fe/0Ni, 1Fe/1Ni, 2Fe/1Ni, 2Fe/2Ni, and 1Fe/2Ni treatments (for explanation see text). Values are means (n=3) with S.E.

Cochlearia pyrenaica ssp. alpina (u. m.)

Fig. 5.1 shows the data for shoot and root dry weights for C. pyrenaica ssp. alpina at harvest after 49 d growth. The control and 2Fe/1Ni treated plants had significantly more shoot tissue than those in the 1Fe/2Ni and 2Fe/2Ni treatments. The difference between the 1Fe/1Ni treated plants and those grown at the higher nickel concentration was also significant. Similar results were obtained for root dry weights.

C. pyrenaica ssp. alpina (non-u. m.)

The control plants had significantly higher shoot and root dry weights than plants in any of the other treatments. No differences were found between treatments with +Ni.

C. officinalis

The highest shoot dry weight was produced by the 1Fe/0Ni plants, about twice as much as by either the 1Fe/1Ni, or 2Fe/1Ni treated ones. The dry weights of the roots showed a very similar trend to those of the shoots. The plants in the other two +Ni (1Fe/2Ni, 2Fe/2Ni) treatments died shortly after the beginning of the experiment and they produced hardly any shoot or root tissue.

CHLOROPHYLL A+B CONCENTRATION OF THE LEAVES

The trend was for chlorophyll a+b concentration to be highest in the 1Fe/0Ni plants.

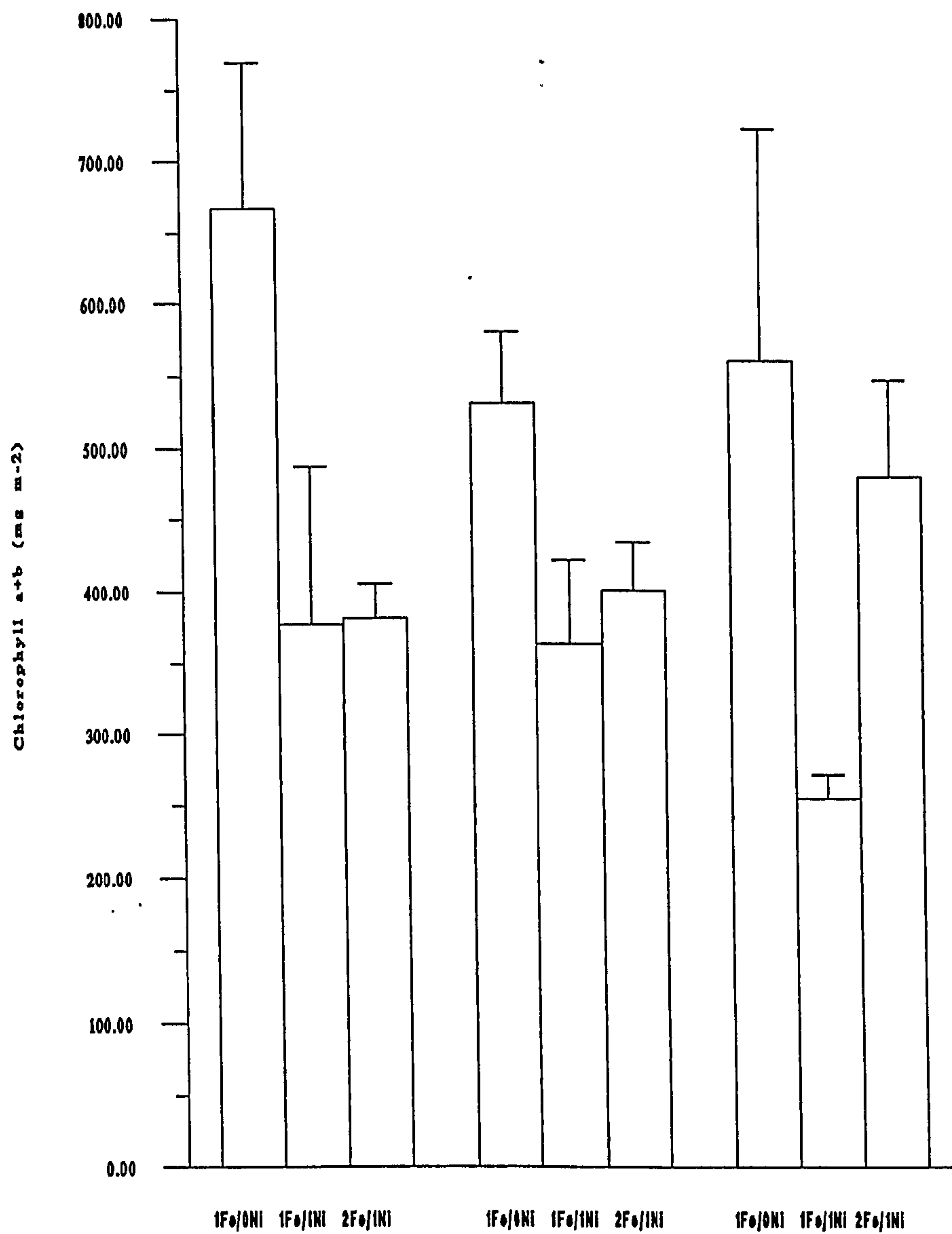


Fig 5.2 Chlorophyll a+b concentration of the laminae used for the polarographic measurement of O₂ evolution in Cochlearia pyrenaica ssp. alpina of ultramafic origin, C. officinalis, and C. pyrenaica ssp. alpina of non-ultramafic origin in 1Fe/0Ni, 1Fe/1Ni and 2Fe/1Ni treatments. Values are means (n=3) with s.e.

There was a reduction in the mean values for 1Fe/1Ni and 2Fe/1Ni in the ultramafic C. pyrenaica ssp. alpina and C. officinalis (Fig. 5.2). The non-ultramafic C. pyrenaica ssp. alpina showed a significant reduction in the 1Fe/1Ni treatment, while the value for the 2Fe/1Ni treatment did not differ significantly from that for the 1Fe/0Ni.

CHLOROPHYLL FLUORESCENCE

The calculated F_v/F_m quotients for the different treatments in the three species are shown in Table 5.2. All mean values were between 0.81 - 0.85, a range generally measured in healthy leaves. The F_v/F_m values were lower in the 1Fe/1Ni in all species. In the non ultramafic C. pyrenaica the 2Fe/1Ni treated plants did not return to the values of those in the 1Fe/0Ni treatment. There was no difference between treatments, or taxa when all three species were included in the ANOVA; 1Fe/0Ni and 2Fe/1Ni for the ultramafic C. pyrenaica differed from 1Fe/1Ni and 2Fe/1Ni for the non ultramafic race at $p=0.12$. (Two individuals of C. pyrenaica (non-u. m.) - one 1Fe/1Ni and one 2Fe/1Ni treated - produced values of 0.74-0.76 and 0.74-0.80 indicating stress damage to photosystem II.)

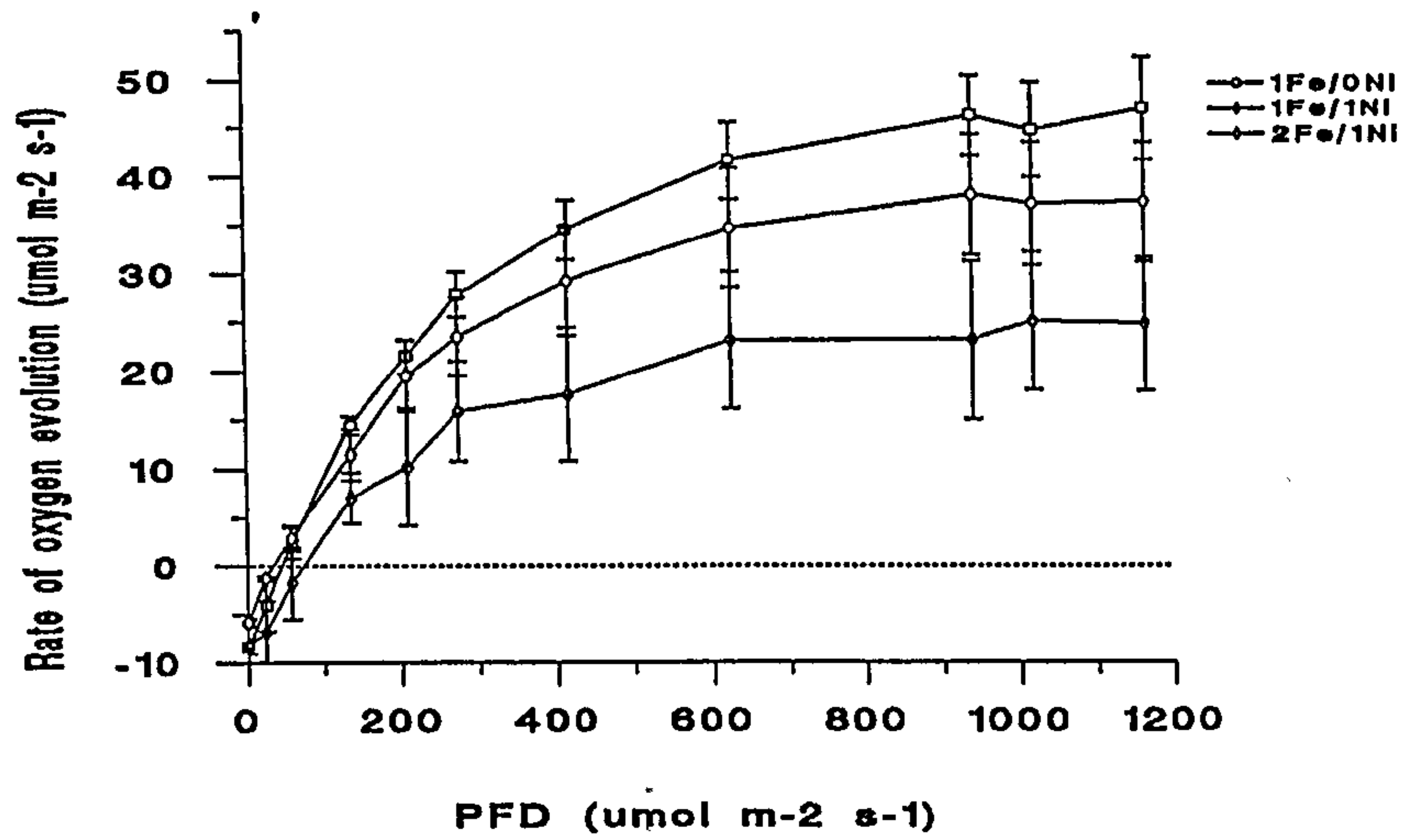
PHOTOSYNTHESIS

Polarographic measurement of oxygen evolution

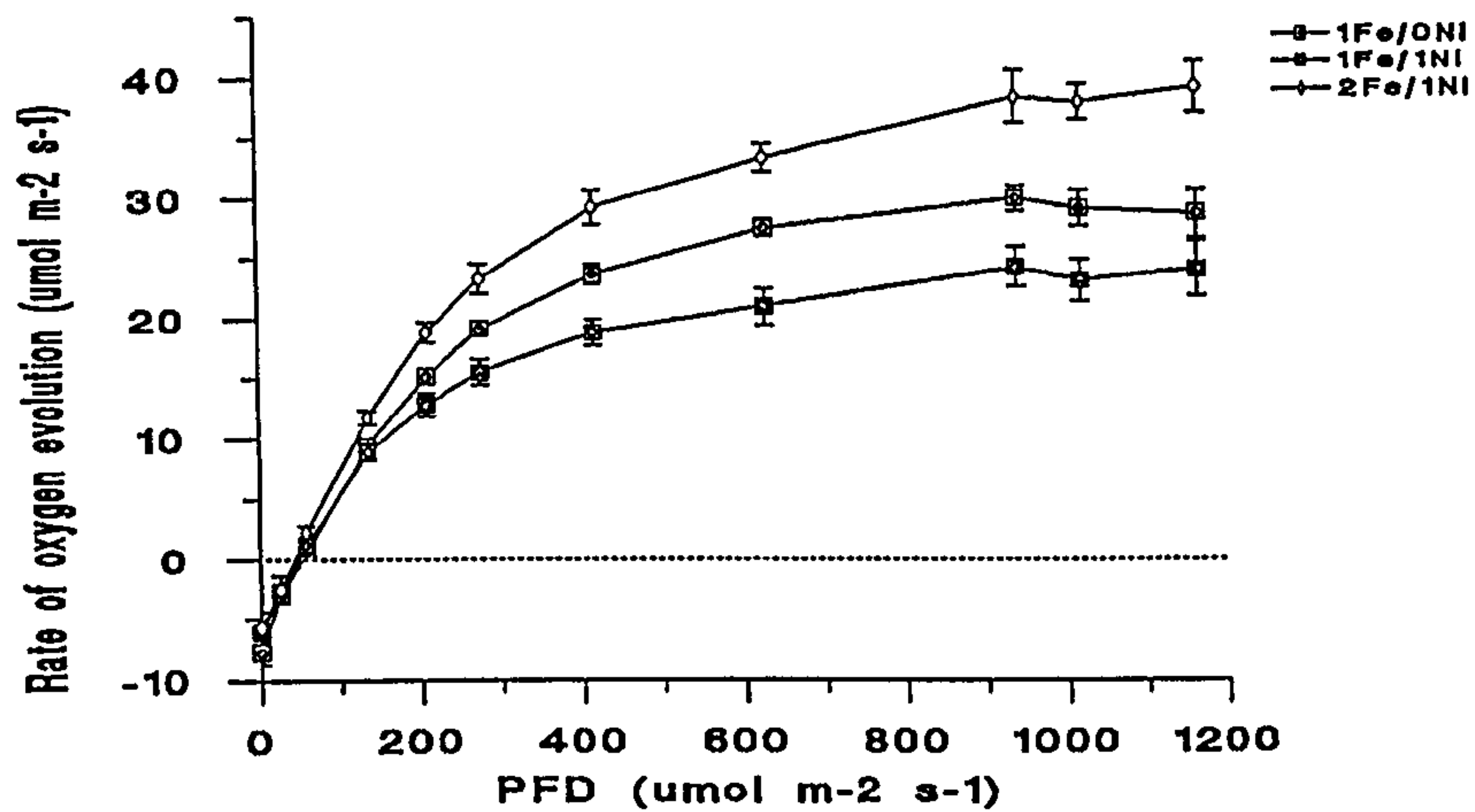
There was no difference in light compensation point. Light saturation was reached at a PFD of about $900 \mu\text{mol m}^{-2} \text{s}^{-1}$ in all three species in all treatments. The maximum rate of the oxygen evolution at light saturation was about $40\text{-}45 \mu\text{mol m}^{-2} \text{s}^{-1}$ (Fig. 5.3).

	<u>C. pyrenaica</u> (u.m.)	<u>C. pyrenaica</u> (non-u.m.)	<u>C. officinalis</u>
1Fe/0Ni	0.843a	0.830	0.842
1Fe/1Ni	0.830b	0.812	0.838
2Fe/1Ni	0.844a	0.810	0.85

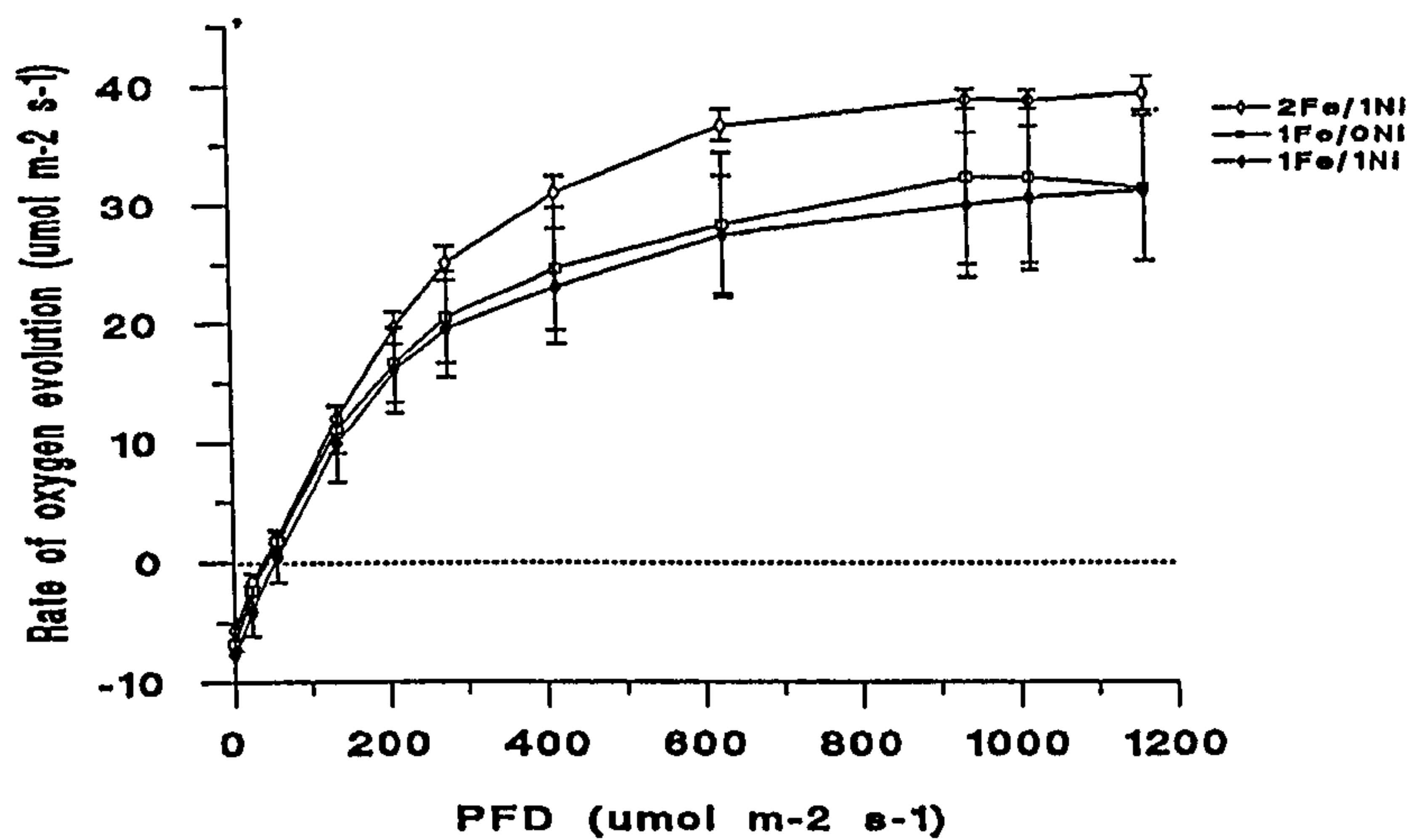
Table 5.2 Values of variable fluorescence (F_v/F_m) measured at 695 nm in the leaves of Cochlearia pyrenaica ssp. alpina of ultramafic (u.m.) and non-ultramafic (non-u.m.) origin and in C. officinalis plants grown in various Fe/Ni treatments. Values are means (n=6); identical letters in C. pyrenaica (u.m.) indicate treatments with no significant difference; there were no differences in the F_v/F_m in the other two species.



(A)



(B)



(C)

Fig 5.3 The rate of oxygen evolution ($\mu\text{mol m}^{-2} \text{s}^{-1}$) versus photon flux density (PFD) in the leaves of *Cochlearia pyrenaica* ssp. *alpina* of ultramafic (A) and non-ultramafic origin (B) and in *C. officinalis* (C) in various Fe/Ni treatments. Values are means ($n=3$) with vertical bars showing s.e.

Statistically significant differences in the rate of light saturated oxygen evolution per unit leaf area between treatments were detectable only in the non-ultramafic C. pyrenaica after reaching light compensation. The increased illumination (136, 209 and 276 $\mu\text{mol m}^{-2} \text{s}^{-1}$) caused a statistically higher rate of oxygen evolution in the 2Fe/1Ni treatment than in the 1Fe/1Ni ($p \leq 0.02$). All treatments were statistically different at higher PFD's with 2Fe/1Ni > 1Fe/0Ni > 1Fe/1Ni ($p = 0.05$).

There were no differences in the rate of oxygen evolution at the four lowest PFD's between the species in the 1Fe/0Ni treatment; thereafter the rate of oxygen evolution in the ultramafic C. pyrenaica was higher than in either the non-ultramafic C. pyrenaica or the C. officinalis ($p \leq 0.10$). No significant differences were found for the 1Fe/1Ni, but there was a consistent order of ultramafic C. pyrenaica > C. officinalis > non-ultramafic C. pyrenaica. Values of photosynthetic oxygen evolution in the 2Fe/1Ni were similar in all species ($p = 0.91-0.99$).

The oxygen evolution of photosynthesis per mg chlorophyll in the different treatments was the same in both races of C. pyrenaica ssp. alpina; in C. officinalis, the values in the 2Fe/1Ni treatment were significantly higher than in the 1Fe/0Ni after the light compensation point ($p \leq 0.05$) (Fig. 5.4).

The species did not differ in the 1Fe/0Ni or in the 2Fe/1Ni while the C. pyrenaica ssp. alpina of ultramafic origin photosynthesised significantly more in the 1Fe/1Ni than that of the non-ultramafic origin at PFD's 136, 209, 276, and 416 $\mu\text{mol m}^{-2} \text{s}^{-1}$ ($p = 0.09-0.033$).

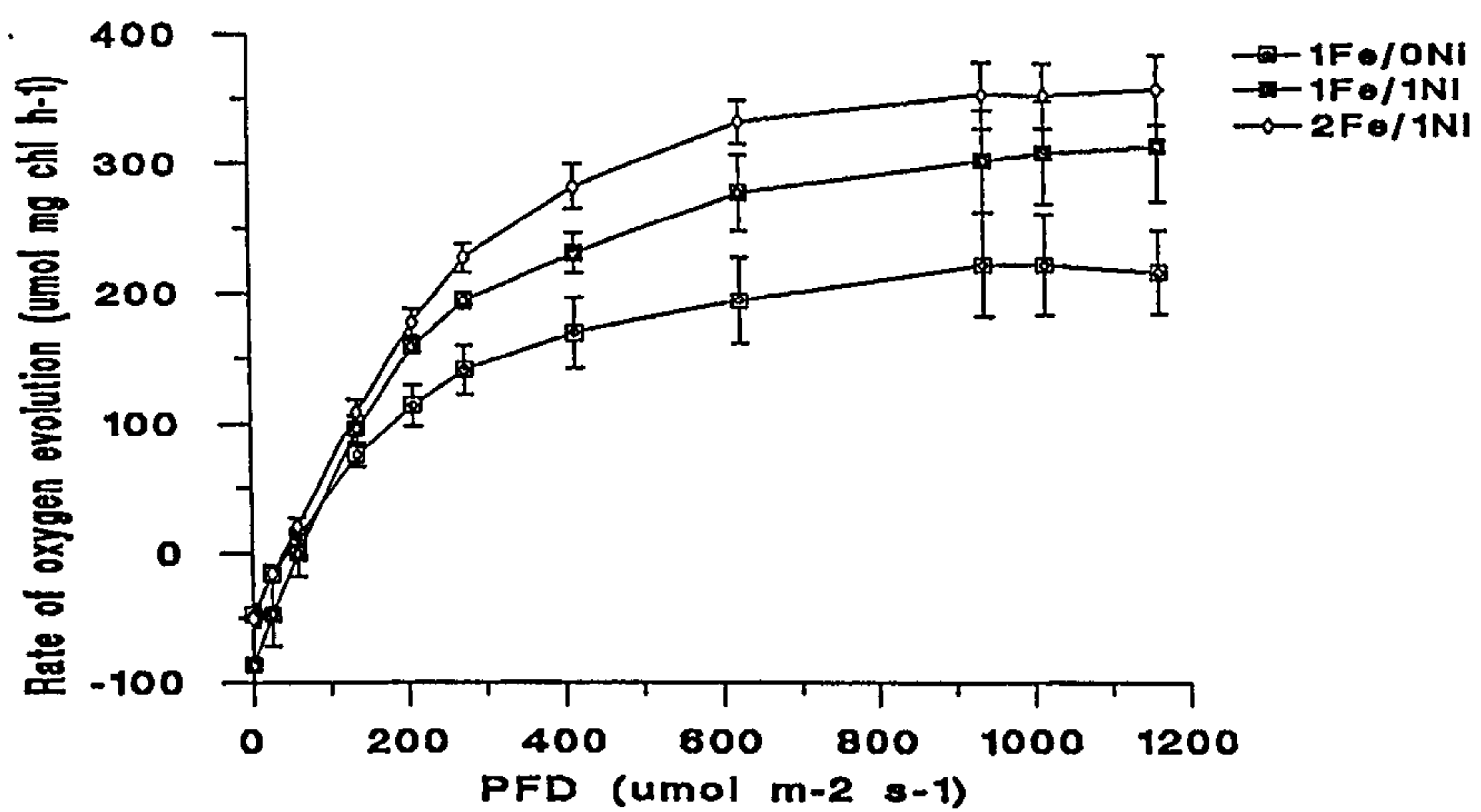
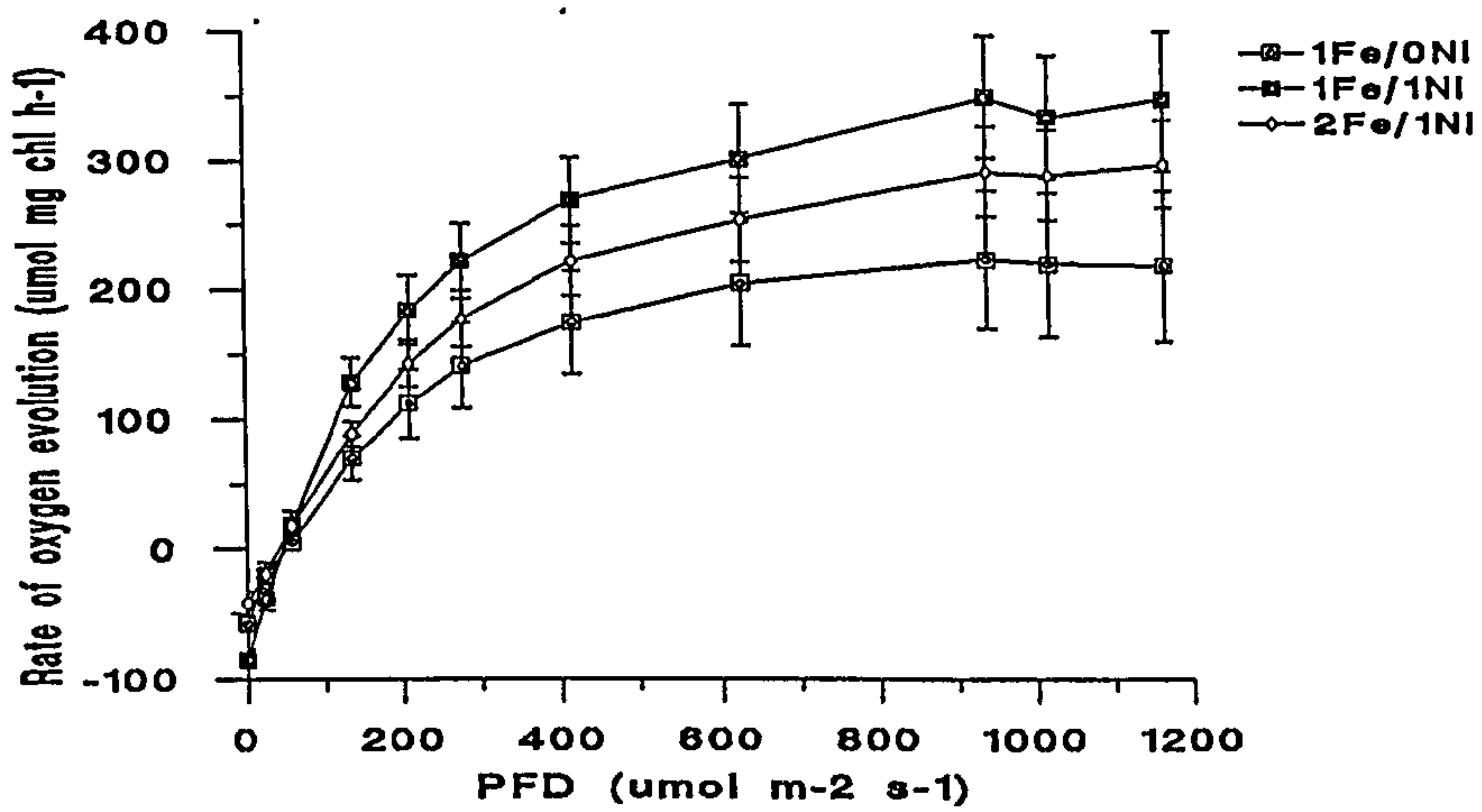
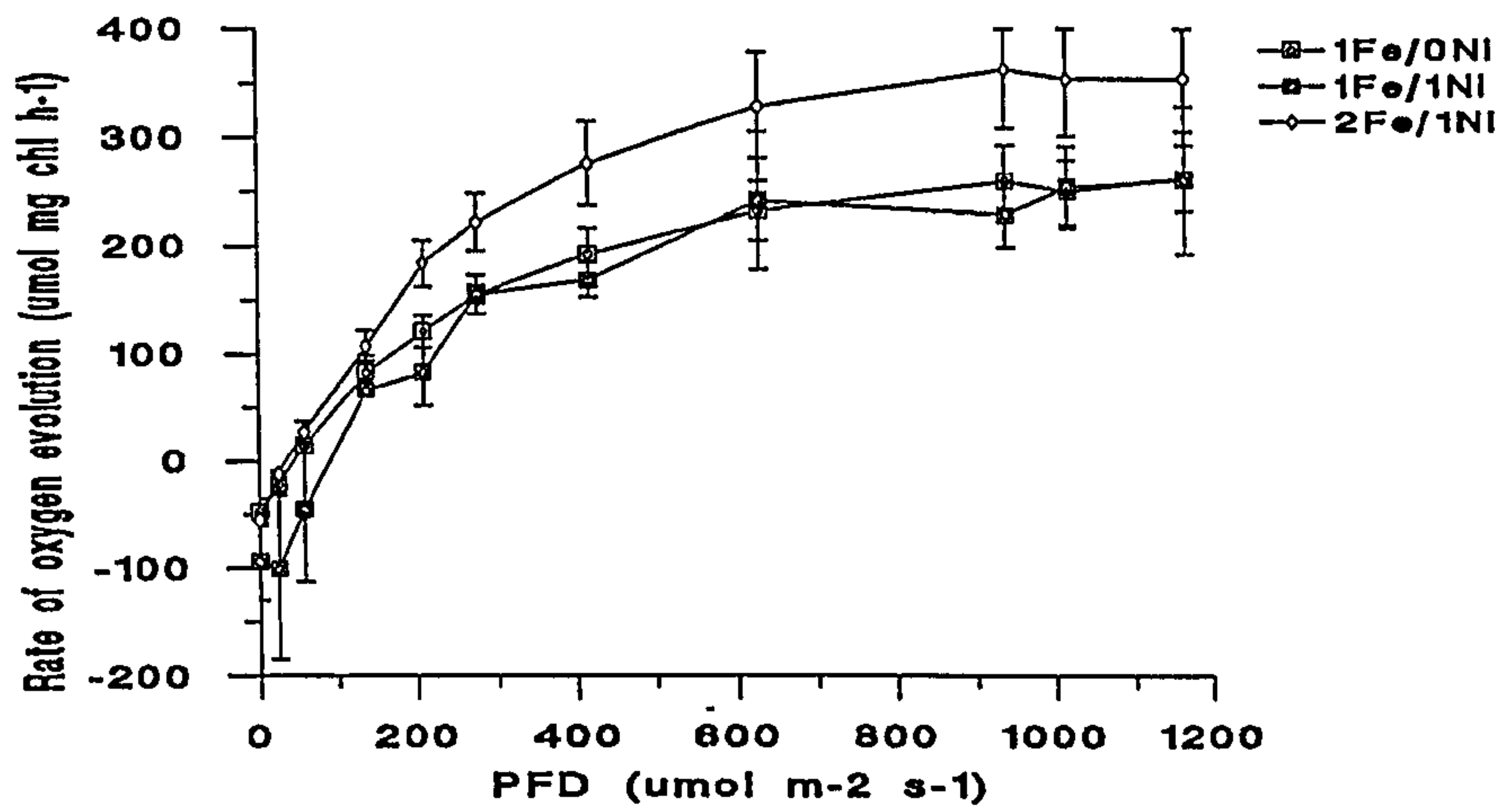


Fig 5.4 The rate of oxygen evolution ($\mu\text{mol mg chl}^{-1} \text{h}^{-1}$) as a function of photon flux density (PFD) in the leaves of *Cochlearia pyrenaica* ssp. *alpina* of ultramafic (A) and non-ultramafic origin (B) and in *C. officinalis* (C) in various Fe/Ni treatments.

Treatment/ Species	Nitrogen ($\mu\text{g g}^{-1}$)	Phosphorus ($\mu\text{g g}^{-1}$)	Potassium ($\mu\text{g g}^{-1}$)	Magnesium ($\mu\text{g g}^{-1}$)	Iron ($\mu\text{g g}^{-1}$)	Nickel ($\mu\text{g g}^{-1}$)
<u>Cochlearia pyrenaica</u> ssp. <u>alpina</u> (u.m.) 1Fe/0Ni	48000	6600	16100	4900	94	-
<u>Cochlearia pyrenaica</u> ssp. <u>alpina</u> (u.m.) 1Fe/1Ni	43400	8000	15600	10900	48	589 (267)
<u>Cochlearia pyrenaica</u> ssp. <u>alpina</u> (u.m.) 2Fe/1Ni	33200	5600	19600	2100*	61	178
<u>Cochlearia pyrenaica</u> ssp. <u>alpina</u> (non-u.m.) 1Fe/0Ni	48900	6400	18700	7500	84	-
<u>Cochlearia pyrenaica</u> ssp. <u>alpina</u> (non-u.m.) 1Fe/1Ni	28000	5900	18600	7800	66	358
<u>Cochlearia pyrenaica</u> ssp. <u>alpina</u> (non-u.m.) 2Fe/1Ni	40000	6200	22600	7500	97	328
<u>Cochlearia officinalis</u> 1Fe/0Ni	29900	3500	10400	8100	54	-
<u>Cochlearia officinalis</u> 1Fe/1Ni	47000	7200	31200	15800*	92	763
<u>Cochlearia officinalis</u> 2Fe/1Ni	46900	5400	27700	12100	116	499

Table 5.3 Chemical composition (dry weight basis) of the laminae of Cochlearia pyrenaica ssp. alpina (u.m.), C. pyrenaica ssp. alpina (non-u.m.) and C. officinalis in 1Fe/0Ni, 1Fe/1Ni and 2Fe/1Ni treatments. *, significantly different.

Treatment	Mean apparent photon yield of photosynthesis (mean \pm SD) (n=3)
<u>Cochlearia pyrenaica</u> ssp. <u>alpina</u> (u.m.) 1Fe/0Ni	0.1046 \pm 0.0088a
<u>Cochlearia pyrenaica</u> ssp. <u>alpina</u> (u.m.) 1Fe/1Ni	0.0830 \pm 0.0089b
<u>Cochlearia pyrenaica</u> ssp. <u>alpina</u> (u.m.) 2Fe/1Ni	0.0886 \pm 0.0209a
<u>C. pyrenaica</u> ssp. <u>alpina</u> (non-u.m.) 1Fe/0Ni	0.0708 \pm 0.0021a
<u>C. pyrenaica</u> ssp. <u>alpina</u> (non-u.m.) 1Fe/1Ni	0.0634 \pm 0.0071a
<u>C. pyrenaica</u> ssp. <u>alpina</u> (non-u.m.) 2Fe/1Ni	0.0880 \pm 0.0061b
<u>C. officinalis</u> 1Fe/0Ni	0.0803 \pm 0.0170a
<u>C. officinalis</u> 1Fe/1Ni	0.0749 \pm 0.0233a
<u>C. officinalis</u> 2Fe/1Ni	0.0874 \pm 0.0092a

Table 5.4 Photon yield for oxygen evolution on the basis of incident photons in the three Cochlearia species in three Fe/Ni treatments. Identical letters for each species indicate no significant differences between treatments ($p \leq 0.05$).

Oxygen quantum yield

The values for mean apparent photon yield were between 0.071 - 0.10. There was a significant reduction in the ultramafic species in the 1Fe/1Ni treatment, while in the non-ultramafic C. pyrenaica the reduced values in the 1Fe/1Ni treatment differed from those in the 2Fe/1Ni, which were significantly higher. There was no difference in the C. officinalis.

ELEMENTAL ANALYSIS OF LEAVES

Table 5.3 shows the results of the chemical analyses of the laminae for the three species in the various treatments. No significant differences were found between treatments in the species apart from magnesium, which was significantly lower in the 2Fe/1Ni treatment in the ultramafic C. pyrenaica than in the 1Fe/1Ni in C. officinalis ($p \leq 0.05$). Foliar iron concentrations in the two C. pyrenaica's were reduced on the addition of nickel to the culture solutions (about 50% in the ultramafic and about 25% in the non-ultramafic). The addition of ten-fold iron (2Fe/1Ni) increased the foliar iron in the ultramafic race to about 66% of its original value in the 1Fe/0Ni, and to about 115% in the non-ultramafic one. In C. officinalis there was no correlation between treatment and solution iron and foliar concentrations of the element. Nickel concentration was also similar in the 1Fe/1Ni and 2Fe/1Ni treatments in this species, while in the two C. pyrenaica's there was a large but not significant reduction in leaf nickel in the 2Fe/1Ni treatment.

Discussion

Many studies have demonstrated that nickel is toxic to plants. In some cases plants of ultramafic origin have been found tolerant of nickel (Johnston & Proctor 1981, Chapter IV this thesis). However, Cochlearia pyrenaica of both ultramafic and non ultramafic origin have been found susceptible to nickel (Chapter IV) and the dry weight values in the different treatments in the study reported here support this. The addition of nickel to the culture solution always resulted in lower plant dry weight, although there were specific differences, with the ultramafic plants being affected to a lesser extent than the other two. The addition of ten-fold iron (2Fe/1Ni) only ameliorated nickel toxicity in the ultramafic plants. Leaf area extension in the ultramafic species was not affected by the two added nickel treatments in which the plants survived (1Fe/1Ni and 2Fe/1Ni) while there was a reduction in both species of non-ultramafic origin. Many of the plants of non-ultramafic origin in the 1Fe/1Ni and 2Fe/1Ni treatments suffered a reduction of photosynthetically active leaf area through areas of necrosis. This was in accord with the findings of earlier studies (Crooke et al. 1954, Vergnano & Hunter 1953). Cochlearia of ultramafic origin also suffered some necrosis in the 1Fe/1Ni treatment, however to a much less extent than its non-ultramafic conspecific. Crooke et al. (1954) argued that necrosis in the leaves of oat plants was related to tissue nickel concentration, and chlorosis was a function of the tissue Ni/Fe quotients.

Eighty percent of the leaf iron is located in the chloroplasts (Marschner 1986) with about 60% as lamellar iron and about 20% in the stroma in plants with sufficient iron. Iron is required for chlorophyll synthesis, its synthesis being restricted by iron deficiency at two stages: the synthesis of δ -aminolevulinic acid, the common precursor

of chlorophyll; and the synthesis of protochlorophyllide. Several authors reported a close positive relationship between iron and chlorophyll concentrations in plants grown at suboptimal iron supply in studies with controlled nutrient supply (Römheld & Marschner 1981, Terry & Law 1982). There appeared to be a reduction in chlorophylls in the +Ni treatments in the present study, but this was not significant. The decrease in the mean concentration of the chlorophylls was proportional to the decrease in leaf iron concentration in both the ultramafic and non-ultramafic C. pyrenaica; the lack of this relationship in C. officinalis may have been caused by the much higher tissue nickel concentration in both 1Fe/1Ni and 2Fe/1Ni.

The F_v/F_m quotients reported here agreed with the average value of 0.832 found by Björkman & Demming (1987) in healthy leaves of a large number of species. Studies investigating the impact on the photosynthetic system of several stresses e.g. temperature and drought showed a decreased F_v/F_m . Values of 0.725 and below measured at 695 nm are commonly regarded as a clear indication of stress damage to PSII (Bolhàr-Nordenkampf & Öquist 1993). No evidence emerged from the present study to indicate that the photosynthetic apparatus of the Cochlearia plants was damaged, though the low values of F_v/F_m measured in two leaves in the C. pyrenaica of non-ultramafic origin suggested some inhibition.

The values obtained for the absorbed quantum yield of photosynthesis are good indicators of the efficiency of the photosynthetic system (Björkman & Demming 1987); the quantum yield of photosynthesis measured as oxygen evolution is about 0.10 (Taiz & Zeiger 1991). No effect of iron deficiency on quantum yield was found at low PFD's by Terry (1980), but he did find an effect at high PFD's. The apparent quantum yield of photosynthesis in the present study was reduced in the ultramafic C. pyrenaica in the

1Fe/1Ni treatment suggesting damage to its photosynthetic system, with the non-ultramafic C. pyrenaica having significantly lower values in the 1Fe/1Ni than in the 2Fe/1Ni. This was not consistent with the results obtained for chlorophyll fluorescence, and in the case of the ultramafic C. pyrenaica may have been owing to the poor state of the two replicate plants.

The principal structural changes caused by iron deficiency are most apparent in the chloroplasts: iron deficient plants have chloroplasts with few thylakoids and the chlorophylls decrease with iron deficiency. (One treatment, the 0Fe/0Ni, was originally set up to serve as a control indicating 'true' iron deficiency, however the plants did not survive the duration of the experiment, therefore no data for the reduction of chlorophylls is available for comparison with results in the other treatments.) PSII contains several iron-containing constituents. The decrease in the thylakoids has been found to involve a decrease in the electron carriers associated with the thylakoids (e.g. Spiller & Terry 1980) and the rate of photosynthesis in iron-deficient leaves decreased per unit leaf area but not per unit chlorophyll (Terry 1980), indicating that the photosynthetic apparatus was still intact. Similarly, there was no reduction in photosynthetic oxygen evolution per unit chlorophyll in plants with lower total chlorophyll in this study.

There was little indication in this study that the efficiency of PSII in the ultramafic C. pyrenaica was affected by nickel. The lower quantum yield of oxygen evolution in the 1Fe/1Ni treatment probably reflects the poor state (unusual for this species) of two of the replicates used for measuring oxygen evolution. The F_v/F_m quotients (Table 5.2) showed no damage to PSII which conflicts with Singh et al.'s (1991) findings for Nostoc, though this may be explained by the differences in the photosynthetic system

of the prokaryote from those of higher plants. Also, it may reflect some adaptation by Cochlearia to high substrate nickel.

The significantly lower rates of oxygen evolution of light-saturated photosynthesis and the two occurrences of values of about 0.74 of the F_v/F_m quotients in the non-ultramafic C. pyrenaica in the 1Fe/1Ni treatment suggest that the applied concentration of nickel may have inhibited its PSII. The inhibition of PSII by nickel in isolated chloroplasts of Ocimum basilicum was clearly demonstrated by Veeranjaneyulu & Das (1982) with the main site of accumulation of nickel in the thylakoid lamellae. The ameliorative effect on the photosynthetic oxygen evolution (significant) and leaf total chlorophyll (not significant) of the ten-fold addition of iron suggested that the hypothesis that there was a competition between iron and nickel which could inhibit photosynthesis was justified. It is noticeable however that this amelioration did not result in plant dry weights similar to those in the 1Fe/0Ni. This meant that the effect of nickel taken up by plants could be ameliorated to some extent in a species-dependent manner, but could not be wholly rectified. A possible explanation is that stomatal regulation and net CO₂ assimilation was affected also by nickel (viz. Carlson et al. 1975). Photosynthetic CO₂ exchange (dark respiration, CO₂ assimilation) was also measured in the present study but the results were difficult to interpret and are not given in detail here.

The aim of adjusting foliar Fe/Ni quotients by varying the concentrations of the two elements in the culture solution was not met and all the results should be interpreted in this light. No conclusive evidence was found in this study to support the hypothesis that sufficient foliar iron can protect the photosynthetic system from the inhibitory effects of nickel. In the meantime the better growth of the ultramafic species than that of the non-ultramafic congeners suggests a higher level of nickel tolerance in the former.

Further investigations are needed into the physiology and biochemistry of nickel tolerance mechanisms in plants. Finally, it must be born in mind that in this type of studies high nickel may affect several sites, others than those associated with photosynthesis, and those other effects may interact with each other and indirectly with photosynthesis and the whole plant responses will be difficult to break down into their components.

Chapter VI

The 'carrying capacity' of MK1

Introduction

The open character of the fellfield type skeletal soils, referred to as 'serpentine debris' by Spence (1957), on ultramafics in temperate climates has long been subject to speculation. Various possible causes, acting alone or in combination, have been put forward which might prevent the development of total plant cover: low soil major-nutrient status; high soil magnesium and low calcium; nickel toxicity; and adverse soil physical factors (Rune 1953, Spence 1957, Proctor & Woodell 1975). Previous short-term work at the Meikle Kilrannoch outcrops has not been able to establish unequivocally whether the areas with low plant cover were predominantly in the building-up (pioneer), or degrading phases of vegetation development (Marshall 1959, Proctor et al. 1992). These authors proposed that the openness was at least partly caused by cryoturbation and soil erosion. However, the problem of low plant cover has never been phrased in terms of 'carrying capacity' and its components determined by site characteristics.

Heeding Dhondt's (1988) caution about the often vague use of the term 'carrying capacity' its use here is now defined. Begon & Mortimer (1986) proposed the following definition: "a particular population level where no increase or decrease occur in population size - an equilibrium point". They pointed out, however, that "no single carrying capacity can ever characterise a natural population: most aspects of the

environment are far too variable, and its own behaviour is never wholly predictable." Taylor *et al.* (1990) defined mean annual carrying capacity as "the mean within-year maximum potential biomass or productivity that the habitat can support in the absence of disturbance". In this study I use the terms 'potential carrying capacity' (henceforth called PCC) to describe 'maximum densities of Cochlearia pyrenaica ssp. alpina and Lychnis alpina individuals grown in pure stands in stone-free soil-surface conditions' and 'realisable carrying capacity' (RCC) for maximum possible plant density (or attainable vegetation) after accounting for the plant 'available surface area' (AA) which is the percentage of stone-free soil area .

The main objectives of the study were to supply data which could be used to characterise the site's PCC and RCC; and to use the data to compare how the vegetation on skeletal soil at MK1 compared with the calculated values of RCC.

Material and Methods

POTENTIAL CARRYING CAPACITY (PCC)

Plant species

Seeds of Cochlearia pyrenaica D.C. ssp. alpina (Bab.) Dalby were collected from MK2 in 1991 and seeds of Lychnis alpina (G. Don) L. from MK1 in the same year and were stored air dry at room temperature. (MK1 seeds were not available for the Cochlearia because they had been used in the experiments described in Chapters IV and V. However MK2 is clearly similar to MK1 chemically and it is unlikely that the different

origin of seeds was of importance in the present study.)

Growth room study

C. pyrenaica ssp. alpina seeds were sown in bulked soil (2 parts from MK1:1 part from MK2) which had been collected from 0-10 cm depth from the sites on 17 November 1990 and stored air-dry for 28 months in a growth room at 15-20 °C. Five 14DX (14 cm diameter) pots were filled with the soil and then the pots were placed into a plastic tray containing deionised water to allow the soil to become thoroughly moist. A seed grid with 1 cm spacing was used for drilling. On 3-4 April 1993 nine rows were sown with ten seeds per row resulting in 90 seeds in a grid in each pot. Watering was with deionised water from below to maintain the soil thoroughly moist.

Plants were recorded individually at weekly intervals, beginning 20 d after sowing, for the following attributes: -, not germinated; G, germinating; E, emerging; S, seedling with cotyledons; and S1 - .. Sn, seedling with a range of numbers of developing leaves.

Field study

The field study was designed to give estimates of PCC as a function of soil chemical (nutrient supplying capacity) characteristics only, with as little interference by physical factors as possible. Three quadrats of 10 cm x 10 cm each were cleared of stones in each of three different areas of MK1 for C. pyrenaica ssp. alpina. The three areas were selected because preliminary results on the population dynamics of L. alpina suggested that two of them had contrasting dynamics of Lychnis (see Appendix I) and the third

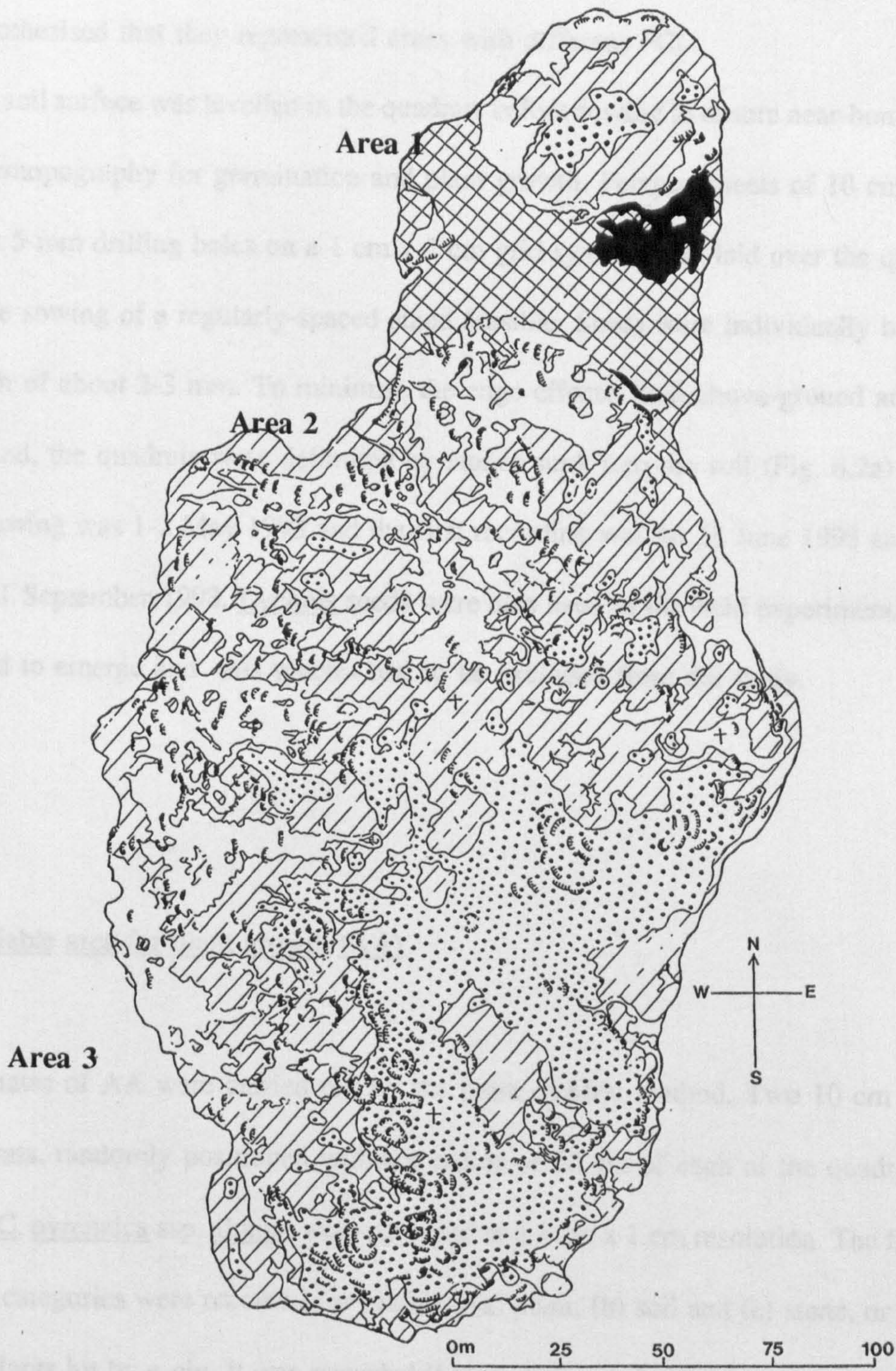


Fig 6.1 Map of MK1 showing the location of areas 1-3 where the estimates of potential carrying capacity (PCC) and realisable carrying capacity (RCC) were made in 1993.

was set up adjacent to the spatial pattern study (Chapter II.) (Fig. 6.1). It was hypothesised that they represented areas with different PCC.

The soil surface was levelled in the quadrats before sowing to ensure near-homogeneous microtopography for germination and plant growth. Perspex sheets of 10 cm x 10 cm with 5-mm drilling holes on a 1 cm x 1 cm grid system were laid over the quadrats to make sowing of a regularly-spaced stand feasible. Seeds were individually buried to a depth of about 2-3 mm. To minimise the edge effects, both above-ground and below-ground, the quadrats were delimited by stones sunk into the soil (Fig. 6.2a) The date of sowing was 1-2 May 1993 and the first recording was on 28 June 1993 and the last on 11 September 1993. Lychnis seeds were also used in the field experiment, but they failed to emerge and this species had to be excluded from the study.

RCC

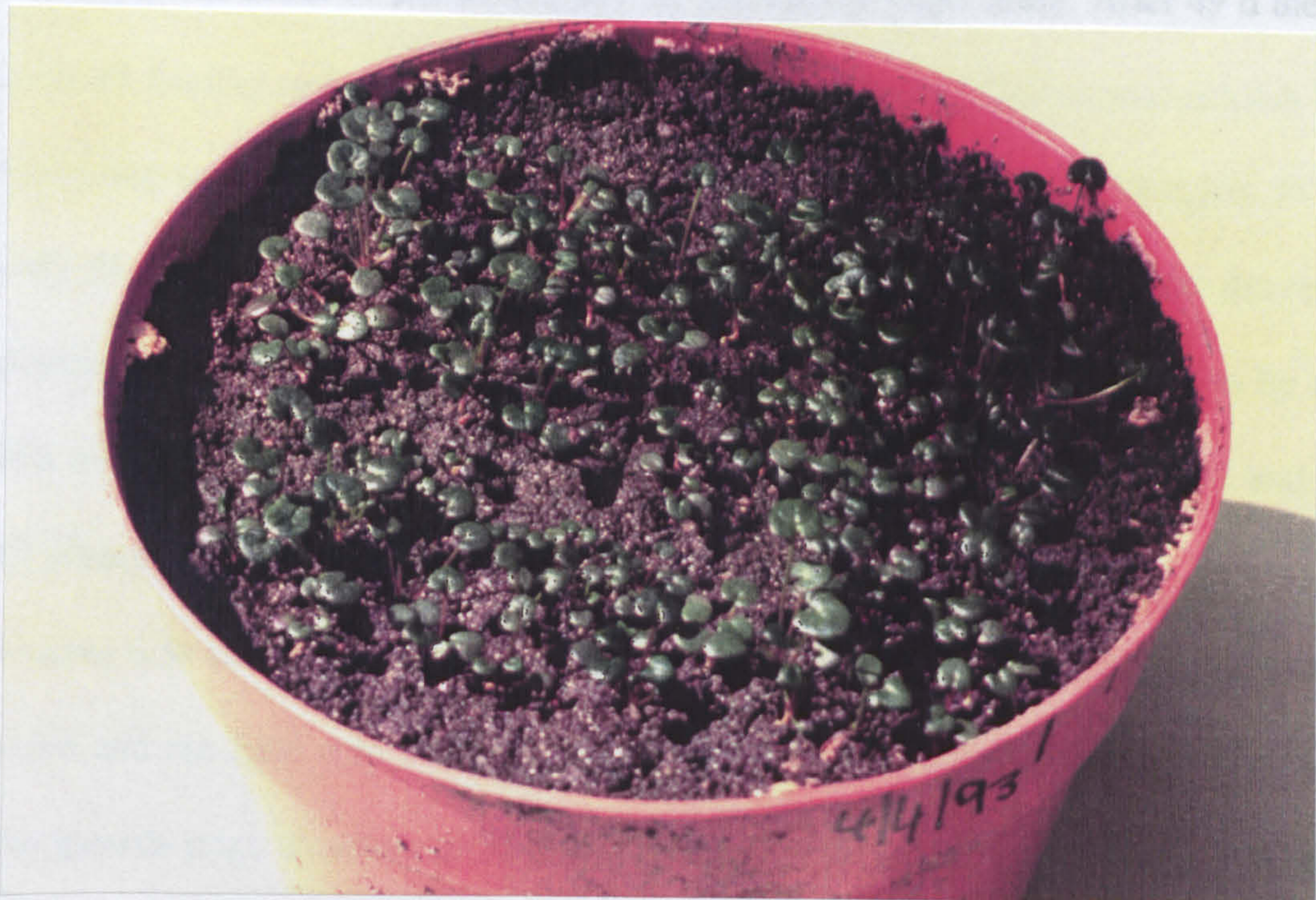
Available area for plant growth (AA)

Estimates of AA were carried out by the point quadrat method. Two 10 cm x 10 cm quadrats, randomly positioned within a radius of 50 cm of each of the quadrats sown with C. pyrenaica ssp. alpina, were surveyed at a 1 cm x 1 cm resolution. The following three categories were recorded for pin hits: (a) plant, (b) soil and (c) stone, or bedrock. For plants hit by a pin, it was recorded if they were overlying soil, or stone, thus both plant cover and available and non-available area were recorded simultaneously.

Estimates of soil:stone volume ratio had also been projected, however, were not completed yet.



(a)



(b)

Fig 6.2 *Cochlearia pyrenaica* ssp. *alpina* in the field study (a, 0.6 x life size) and in the growth room study (b, 0.85 x life size) after 49 d growth.

RCC

RCC was calculated as $PCC \times \text{mean AA (\%)} / 100$. The values recorded for plants in the two quadrats in the vicinity of each of the sown quadrats were used to compare the results calculated for RCC.

Results

PCC

The PCC was about 77 plants 100 cm^2 in the growth room study. After 49 d the plants were all healthy and green (Fig. 6.2b) but after about 63 d they turned reddish-brown, indicating some stress. In one pot which became inadvertently waterlogged, PCC was only 24 plants 100 cm^2 , and was not included in calculating the mean for the rest. The experiment had to be terminated after about 70 d because of an infestation by aphids. The average PCC in the field trial was about 36 plants 100 cm^2 in areas 2 and 3 and 55 plants 100 cm^2 in area 1.

Figures 6.3a and 6.3b compare the growth-stage structure of the seedlings in the growth room and the field trial after 49 d. It can be seen that there was a large difference in the growth-stage structure irrespective of whether values are expressed as a percentage of the number of seeds sown, or as a percentage of total plants established. While all but 6% of the pot-grown plants in the growth room study passed the two-leaf stage 49 d after sowing, in the field only 5-10% of emerged plants grew two foliage leaves (none

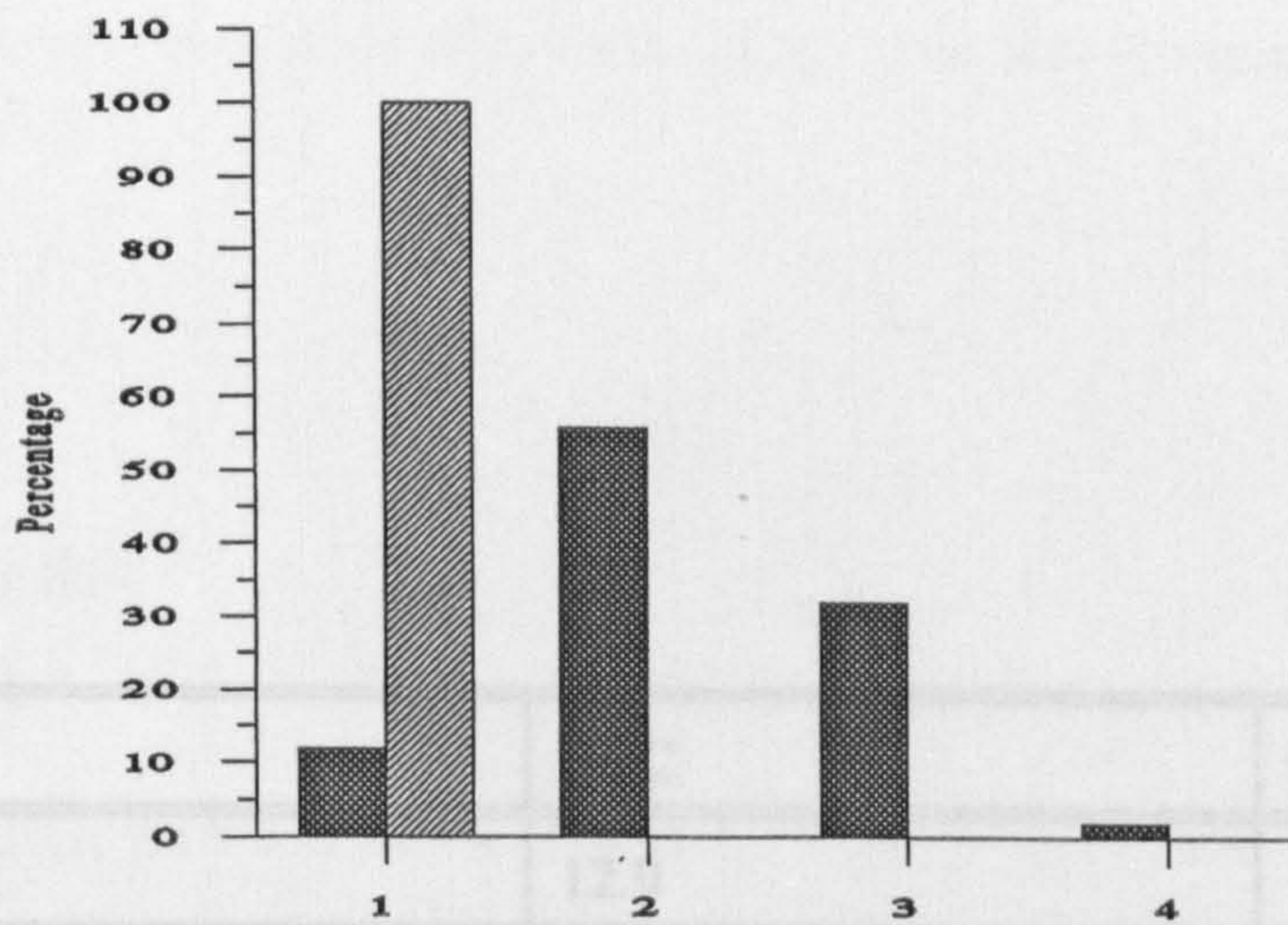


Table 6.1 Relative carrying capacity (RCC) and real cover based on the point quadrat method at MKI 1993. Values for RCC are means of three 10 cm x 10 cm quadrats in each of areas 1-3 described in the text. Real cover values are means of six 10 cm x 10 cm quadrats in each area. For calculation of RCC see text.

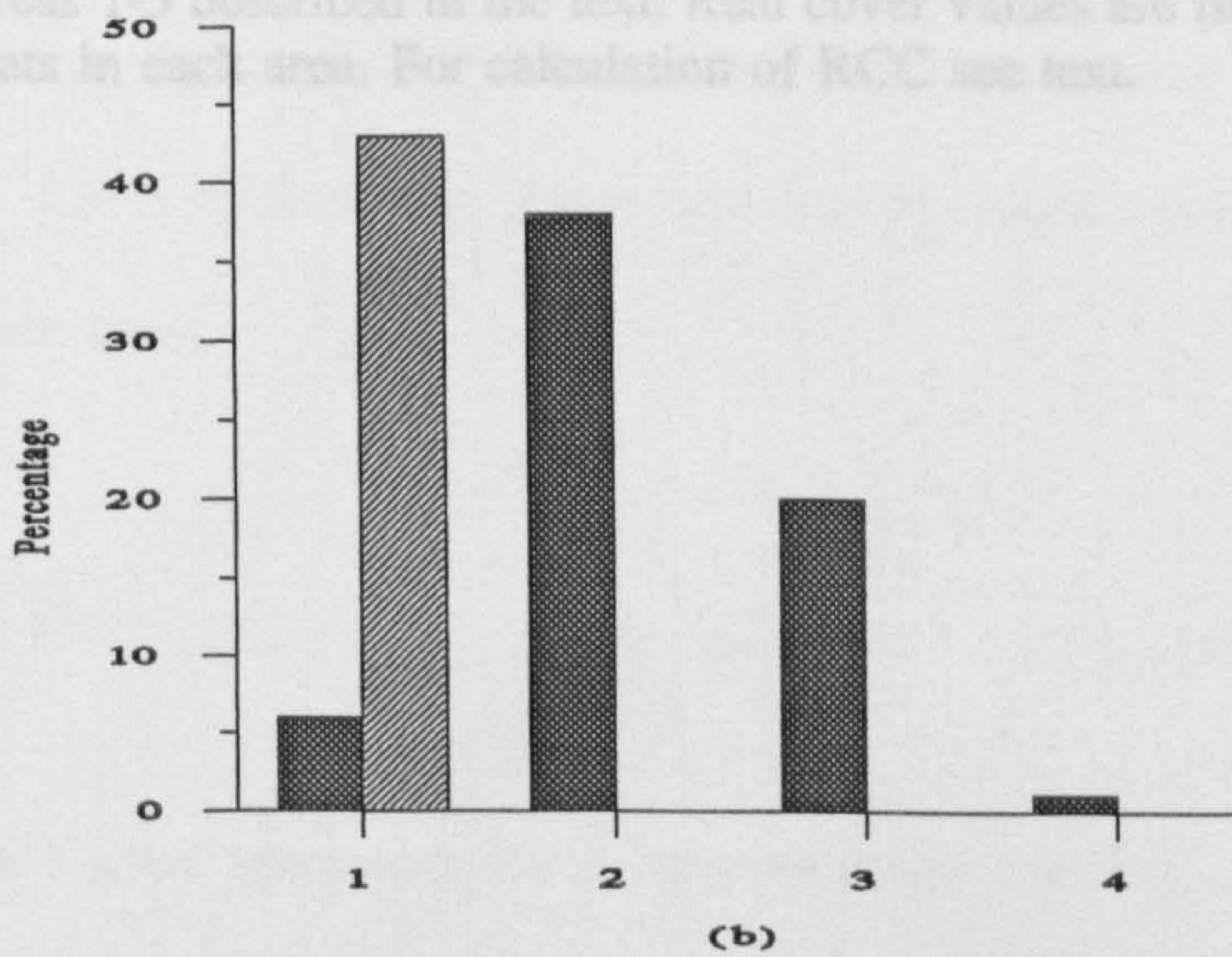


Fig 6.3 Phenological stage (1: 0-2; 2: 2-4; 3: 4-6; and 4: > 6 leaves) distribution (%) of *Cochlearia pyrenaica* ssp. *alpina* after 49 d growth in a growth room (filled bars) and in the field (cross-hatched bars). values are percentages of total plants (a) and of seeds sown (b).

	RCC	Real cover %
Area 1	12.9	12.8
Area 2	6.6	9.3
Area 3	16.9	8.3

Table 6.1 Relative carrying capacity (RCC) and real cover based on the point quadrat method at MK1 1993. Values for RCC are means of three 10 cm x 10 cm quadrats in each of areas 1-3 described in the text. Real cover values are means of six 10 cm x 10 cm quadrats in each area. For calculation of RCC see text.

had more), and about 40-50% of them had only cotyledons and one leaf only. The growth-stage structure of field plants after about 140 d growth became similar to those in the growth room study after 49 d, though their size remained much smaller.

RCC

Available area

The available area was 23.9% in area 1, 17.2% in area 2, and 44.5% in area 3. Area 3 had significantly more soil surface for plant growth. Soil depth was significantly shallower in area 2 (where the Lychnis population dynamics study had shown no changes in the species' dynamics over a two-year period as described in Appendix I). Plant cover (including bryophytes, mainly Racomitrium lanuginosum) was the lowest at the southern tip in area 3 (5.7%) and highest at the northern end of the site in area 1 (22.8%). Cover by flowering plants excluding bryophytes ranged from 5.7% - 12.1% for the same areas.

Estimated RCC

There was a good agreement found between calculated RCC values and the existing vegetation cover estimated by the point-quadrat method (Table 6.1).

Discussion

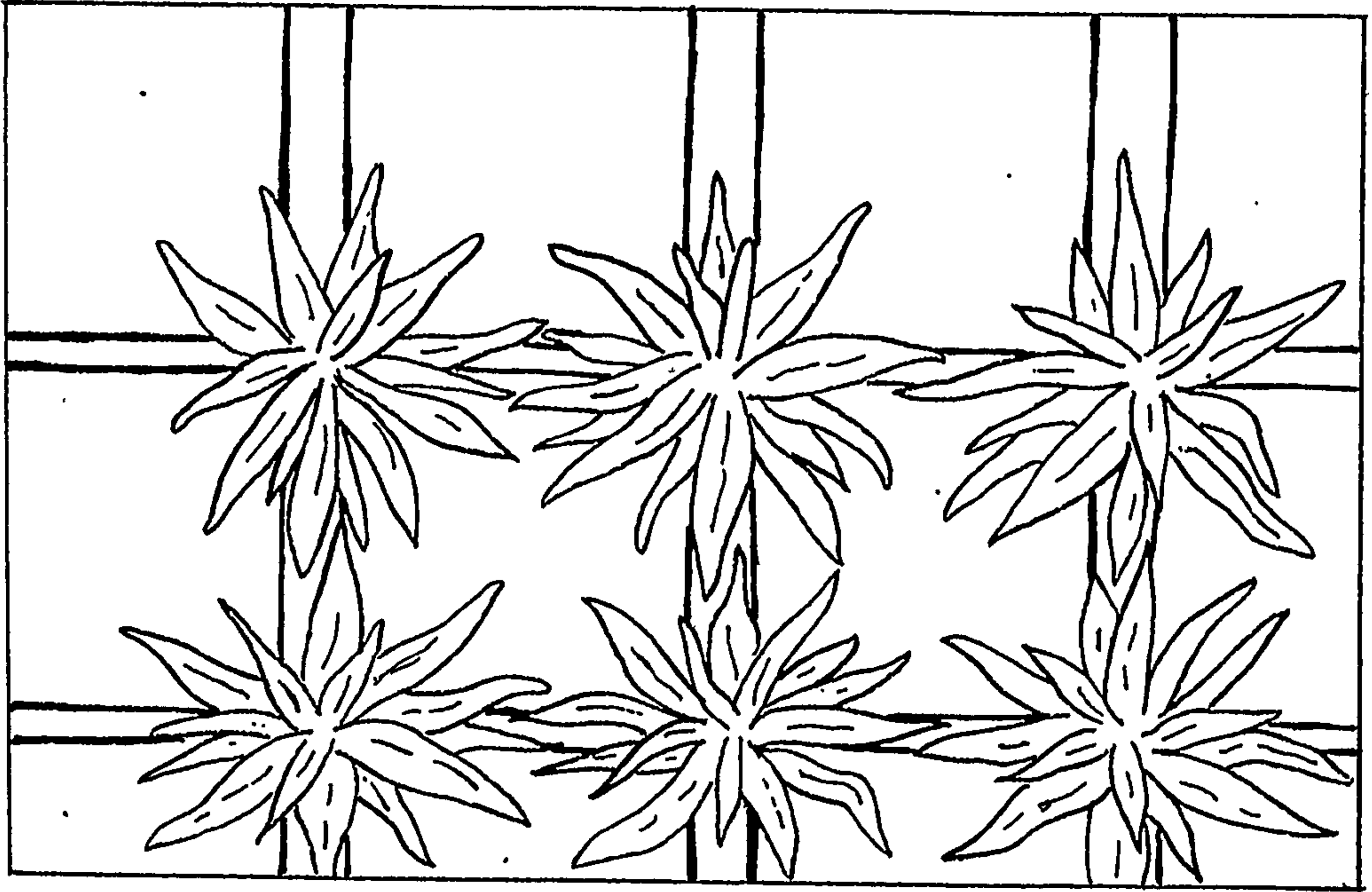
This study was intended as a pilot phase for more broadly-based investigations on the carrying capacity of the skeletal soil at MK1 with regard to environmental factors and plant-to-plant interactions.

There were large differences in the PCC in the pot trial and the field experiment. It is not only that the number of seedlings established was significantly higher in the growth room but also there was a significant difference in growth. On emergence, plants in the growth room achieved larger sizes than those in the field and had a healthy green colour as opposed to the red-brown in the field (Fig. 6.2a,b). All these differences may have been caused by temperature differences alone but it is likely that they were caused by better nutrient dynamics of the soil, probably as a result of the higher temperatures and phosphorus mineralisation during storage. Temperature may have also influenced the uptake of nickel as had been shown for cadmium, lead and zinc which were taken up at higher concentrations at lower temperatures by barley bioassay plants and also by several native species in two types of vegetation in Upper Teesdale (Waughman et al. 1983). The later change in the colour of the pot-grown plants, resulting in the same coloration as that prevailing in the field, suggests that they became limited by phosphorus, or other nutrients. It is unfortunate that the aphid infestation prevented the experiment from being completed and hence also the calculation of the PCC of the MK1 ultramafic soil as a function of soil volume available for plant root growth. An inadvertently imposed waterlogging in one of the pots resulted in a very low emergence of C. pyrenaica ssp. alpina. As extensive areas of MK1 and MK2 are affected by a periodically high water table, experiments should be made to quantify the possible

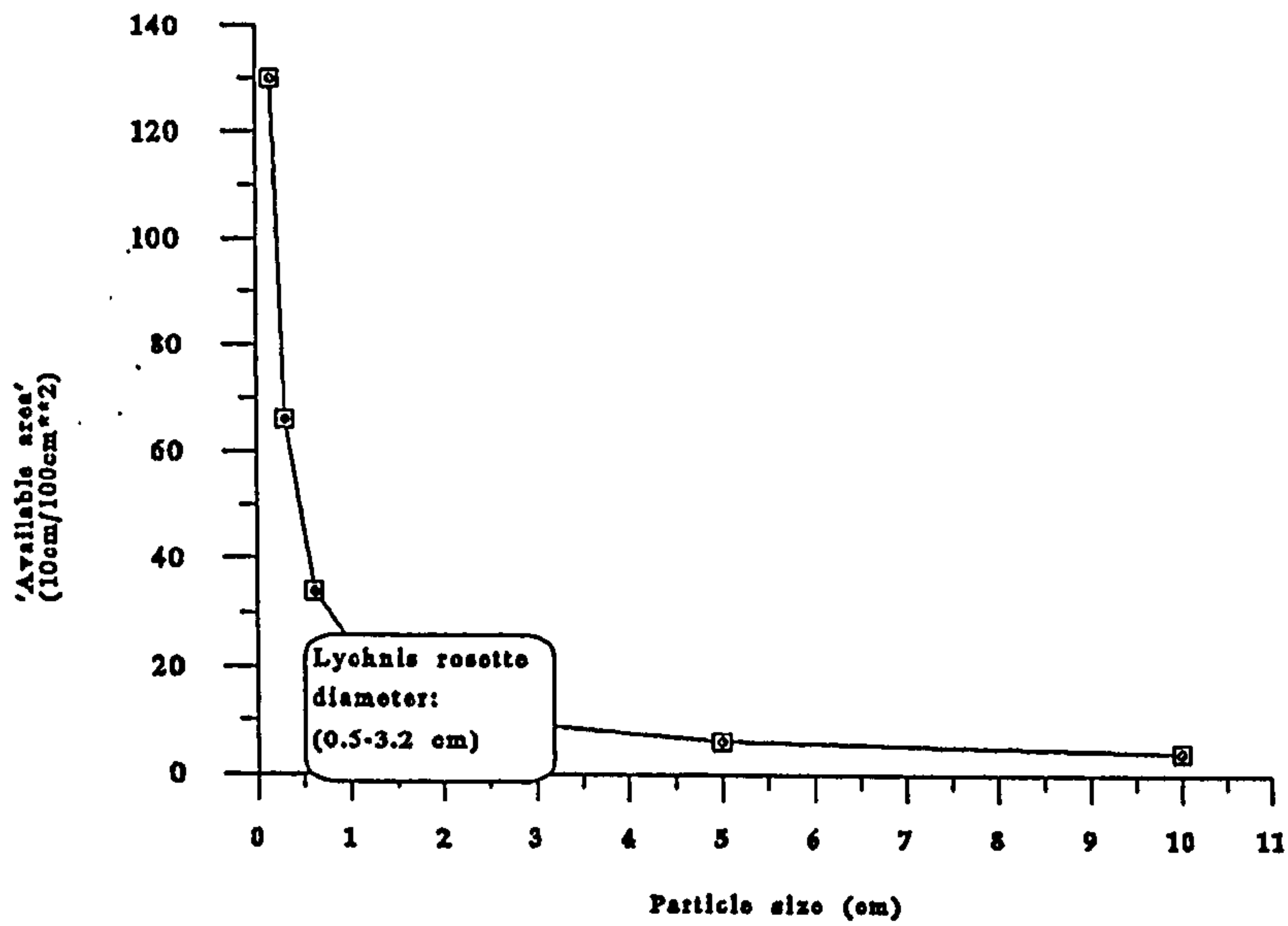
effects of waterlogging on seedling emergence, establishment and later growth in relation to potential carrying capacity.

The calculated RCC's were in good agreement with the measured cover for all but one area. Available area, defined as available resource area, has been the subject of a number of studies investigating population density regulation (e.g. Watkinson *et al.* 1983, Owens & Norton 1989). In the present study, available area was considered as available physical space in a first approach to divide space into stones (non-available area) and soil (available area). The proportion of available to non-available area is low: that is surface fragmentation (stone size) may itself be a factor restricting vegetation development as is shown in Fig. 4. Available area was calculated as described in Chapter II and data for Lychnis were also collected in that area. If stone size were not limiting, Lychnis could in theory reach a total cover over the area (78.8% - assuming no overlap between neighbouring rosettes - Fig. 6.4a). This could be attained if average rosette diameter equalled average stone size as shown in Figure 6.4b. Larger stone size would however prevent the species from attaining the theoretically possible maximum cover. Data collected for Lychnis rosette sizes for all 228 10 cm x 10 cm quadrats in the pattern study in June 1993 showed that the size of the stones itself can prevent this species reaching full cover over some areas of MK1 (Fig. 6.4b).

Available resource area, which can be defined as a function of available physical space and plant-to-plant distances, is also of major importance. Studies elsewhere have shown that reduced available resource area reduces seedling survival (e.g. Owens & Norton 1989). In the present situation, reduced available resource area is of great importance as flowering specimens of species growing at MK1 are widely spaced (15-20 m² for example for Cochlearia and 15-30 m² for Lychnis) and they have restricted seed



(a)



(b)

Fig 6.4 Illustration of maximum cover by rosette forming plants over areas with angular stone cover (a). The range of rosette diameters in *Lychnis alpina* at MK1 in relation to 'available area' as a function of surface stone size (b). In areas with stone sizes > 3.2 cm *Lychnis* cannot reach maximum cover.

dispersal. This will result in high local output of seeds creating a highly competitive environment for seedlings with low available resource areas. This, in theory, should result in high seedling mortality (Antonovics & Levin 1980). Observations at the site support this for Lychnis (Appendix I) but not for Cochlearia, where, it seems, most emerging plants will establish. On the other hand many fewer Cochlearia than Lychnis emerge which may be caused by the susceptibility of the former to damping-off (Hussein 1994).

Although the projected estimation of the soil:stone volume ratio, to make a comparison possible for plant growth as a function of available soil volume, was not completed, it is reasonable to assume that the available area as physical space is also restricted below ground. This has probably more profound effects on controlling PCC than available surface area. The studies by McConnaughty & Bazzaz (1991, 1992) in which they investigated the effect on plant growth of fragmentation of physical space, by using artificial root systems in their pot trials, are relevant. They found that the fragmentation of space caused reduced plant growth in a specific dependent manner and independent of water availability. Nutrient addition counter-balanced reduced plant growth caused by fragmented space. (Campbell *et al.* (1991) also noted that their results on simulating nutrient patchiness without restricting physical space conflicted with those of Crick & Grime (1987), where solid partitions were used to separate areas with different nutrient availability.) The high stone content of the soil at MK1 will similarly constrain physical space and, in addition, reduce rooting volume. This has consequences on the efficiency of roots' ability to obtain nutrients and can much increase the overlap of nutrient depletion zones around the roots. Potassium and phosphorus, of which the latter has been shown to be a controlling factor for plant growth at the nearby MK1.5 site

(Chapter VII), can especially be affected (Newman & Andrews 1973, Nye & Tinker 1977, Fitter 1987). No root architecture investigations have been made at Meikle Kilrannoch but Spence's (1957) work on the ultramafics of Unst, Shetland may be used as a pointer. He found that although above-ground plant distances were high (cover was 5-10%), the roots of the plants extended to 15-30 cm around the plant and overlapped. Spence suggested that "competition for space can exist even at the lowest covers". This will undoubtedly be true as long as the soil depletion zones for water and nutrients around the roots also overlap.

Different specific responses to fragmented and restricted space as reported for four weed species by McConnaughty & Bazzaz (1992) may exist at MK1 and can have implications for the species' ability for below-ground competition for space and resources.

The MK1 habitat falls into the category of habitats with disturbance and high stress, providing marginal conditions for plant life in Grime's (1979) classification. The species make-up accordingly should consist of species of the 'stress tolerator' functional type. The C-S-R model is essentially concerned with the conditions of co-occurrence of the three main functional types and the implications for vegetation dynamics at a given scale. Modelling, based on the original C-S-R model, (Colasanti & Grime 1993) does not provide for situations such as the present one, where interactions occur only between component species of the same functional type. However, the level of interactions (competition) can be of fundamental importance in determining vegetation processes there. Taylor *et al.* (1990) maintain that the intensity of competition should not necessarily decrease in habitats with decreasing resource availability. It seems that their approach is more applicable at the present scale than Grime's broader-based view

that competition gives way to stress tolerance in a nutrient restricted (stressful) environment (Grime 1979, 1988). It is hoped that a field-based series of experiments, called for by Taylor et al. (1990), are to be made on the vegetation development processes at Meikle Kilrannoch together with the experimental characterisation of the carrying capacity.

The above substantiates Klinkhamer's (1989) work on modelling density regulation in sparse populations. The deterministic model proposed by Klinkhamer derives population density from the general growth model where the net rate of increase is derived from available seed number and safe-sites (or available area as physical space) with the assumption that seed dispersal is random. Important deductions include that the number of safe sites occupied can be less than the number available, that is both the available area and the number of seeds can be limiting for population size. Sparse populations, on theoretical grounds, therefore exist at densities below the RCC of the site (sensu Taylor et al. 1990). The number of safe-sites is directly associated with the site RCC and the number of seeds available is probably best defined as a function of plant species and environmental conditions, or in other words the plant genotype expressed as a function of the level of saturation of the carrying capacity. (See also Klinkhamer et al. (1992) on size-dependent seed production.) Reduced seed production in Cochlearia owing to phosphorus limitation has been proposed as a controlling factor for vegetation development on the MK1.5 site (Chapter VII). This, with the restricted available area may be partly responsible for the open character of the ultramafic outcrops at Meikle Kilrannoch.

It should be reemphasised at this stage that this study has been intended to investigate the scope of research on the topic at MK1 rather than produce conclusions. There

remains a list of questions to be answered e.g.: is there a RCC defined by the below-ground availability of resources for plants? Is it reflected in root spatial architecture in exploring and exploiting soil resources? To what extent do roots experience (if at all) a 'wall effect' arising from the physical fragmentation of below ground space? Is there density-dependent regulation, or does the site still offer space for further colonisation by the species already present?

The approach of this pilot study so far has been oversimplistic. It used only one species to estimate carrying capacity. The equation applied to derive RCC is also an oversimplification as it assumes a linear relationship between PCC and AA. It is the scope of further work to refine the methods of estimating carrying capacity and eventually offer a model for monospecific and multispecies situations.

Chapter VII

Demographic, life history, and carbon partitioning responses of Cochlearia pyrenaica D.C. ssp. alpina (Bab.) Dalby to nutrient addition at MK1.5

Introduction

Why make a nutrient addition study? It is often assumed that differences in vegetation are caused by variation in edaphic factors (Greig-Smith 1987) and particular deficiencies, or excesses of soil chemistry are important causes of vegetation, as exemplified by the ultramafics in the present study. Nutrient, or in a broader sense, resource addition studies have proved a useful tool to identify factors which were maintaining characteristic vegetation, or limiting vegetation development processes.

Resource addition experiments are a special type of the more broadly applied perturbation studies carried out to attempt to explain causal factors for regulation processes in vegetation. They are mostly used in both man-made and naturally established stands of plants, with direct applicability for agricultural production. Few nutrient addition studies have comprehensively investigated the regulatory effects of ameliorated resource availability on vegetation development processes in natural vegetation however (DiTommaso & Aarssen 1989), especially on ultramafics (Proctor & Nagy 1992).

The experiments reviewed by DiTommaso & Aarssen (1989) have assessed changes

in: (a) production; (b) species composition; (c) species diversity; (d) neighbour interactions and succession; and other specific purposes. Results in most cases have shown an increase in overall productivity, and changes in species composition with lower diversity after fertiliser application. The analysis of neighbour interactions has revealed that species responded differently to nutrient addition. Fertiliser addition in nutrient-poor habitats such as tundra communities had less pronounced responses (Chapin & Shaver 1985). Nutrient addition experiments have made contributions to the study of the population dynamics of plants (Harper 1977) although few have related to the effects of soil nutrient amendments on the demography of species in natural vegetation (Noble *et al.* 1979, De Jong & Klinkhamer 1988). Nutrient addition experiments are also of interest for plant physiological ecologists who are mainly concerned with the physiological changes prompted by altered nutrient supply in the ecology of individual species which in turn may explain the compositional changes in species assemblages.

A central question for the ecology of ultramafics has been the cause of the low plant cover which occurs in many of them. Since the work of Gordon & Lipman (1926) several workers have held that low concentrations of major nutrients, particularly those of nitrogen and phosphorus cause sparse vegetation. There have been many reports of low concentrations of major nutrients in ultramafic soils (e.g. Minguzzi & Vergnano (1953), Walker (1954), Spence & Millar (1963), Sarosiek (1964), Griffin (1965), Proctor & Woodell (1971), Jones *et al.* (1977), Koide *et al.* (1987)) although others have found no major nutrient deficiencies (e.g. Pelisek 1939, Ishimoto 1958, Carter *et al.* 1987b). The pilot work on the carrying capacity of MK1 showed that the surface stone cover may itself prevent the development of a total plant cover (Chapter VI). Soil

analyses suggested major nutrient limitation as another controlling factor.

In the present study the effects of the addition of calcium and NPK alone, or in combination were investigated at MK1.5.

It was hypothesised that nutrient limitation caused decreased production of assimilates which affected physiological processes and life history at the individual level with consequences for population dynamics and vegetation development. The population dynamics of Cochlearia pyrenaica ssp. alpina and other species were assessed together with concurrent overall vegetation developmental processes. Life history (maturing and flowering) and physiological phenomena (carbon allocation patterns) were also studied. The main objectives were: to test the hypothesis that the underlying cause for low plant cover was the limited availability of nutrients; and identify the critical vegetation development processes which were limited by the prevailing soil and climatic conditions. A secondary aim was to use the data obtained for conservation management.

Material and methods

SPECIES STUDIED

The principal species studied was Cochlearia pyrenaica ssp. alpina (Brassicaceae). C. pyrenaica ssp. alpina is a rosette-forming biennial, or more frequently semelparous winter-green perennial with simple, or branching flowering stems. Iteropary has been occasionally observed in the MK1.5 population. About 1% of reproducing individuals were found to remain alive after seed production and produced new rosettes from branches of the rootstock in the following year. Flowers are 5-8 mm in diameter, petals

are white or pinkish; and an apetalous form was also observed. Fruits are variable, up to 5 mm long with fine-reticulate walls. Seeds are 1.3 - 1.8 mm.

Other species recorded in the experimental quadrats were: Agrostis vinealis, Cerastium fontanum ssp. scoticum, Festuca rubra, Juncus trifidus, Scirpus cespitosus and Huperzia selago and moss species Ceratodon purpureus, Funaria hygrometrica, Racomitrium fasciculare, R. lanuginosum and Splachnum sphaericum.

FERTILISER APPLICATION

Fertiliser rates and the time of application were similar to those in a nutrient addition experiment on the Keen of Hamar, Shetland by Carter et al. (1987a). Nitrogen was supplied as NH_4NO_3 ($100 \text{ kg ha}^{-1} \text{ N}$), phosphorus as NaH_2PO_4 ($50 \text{ kg ha}^{-1} \text{ P}$), potassium as KCl ($100 \text{ kg ha}^{-1} \text{ K}$) and calcium as CaCO_3 (1000 kg ha^{-1}). Nitrogen, phosphorus and potassium were applied in 600 ml solution per quadrat, while calcium carbonate was applied as a powder, on August 3 1991 and July 28 1992. Controls and +Ca received an identical amount of water from the same local source used for +NPK and +NPKCa. A separate small-scale experiment was set up in June 1993 to separate the effects of the major nutrients applied as a combined NPK treatment in the main experiment. NH_4NO_3 , NaH_2PO_4 and KCl were each applied separately at the same rates as those described earlier to three replicated 25 cm x 25 cm quadrats. The quadrats were assessed in June and September 1993 for number of individuals and phenological stage.

POPULATION DYNAMICS

Twelve quadrats each of 0.21 m² (50 cm x 42 cm) were set up in three blocks in May 1991 to study the effects of Ca, CaNPK and NPK addition on the population dynamics of C. pyrenaica ssp. alpina. A metal frame, mounted on legs with individually adjustable height to correct for the effect of slope, was used to hold a rigid Perspex sheet with overhead projector transparencies to mark the position of each individual at each recording (Fig. 7.1). Plastic pegs driven into the soil were used to ensure that the frame was placed over the quadrats in an identical position at each census. The first recording of the plants was on 28 May 1991, before the first fertiliser addition. Recordings were done at monthly intervals during the growing seasons (May to September) with the last recording on 30 June 1993. Herbivory damage was apparent in some of the fertilised quadrats, which had responded to +NPK and +NPKCa with increased growth by October 1991. Exclosure cages were installed in December 1991 to prevent further grazing by mountain hare (Lepus timidus), deer (Cervus elaphus) and ptarmigan (Lagopus mutus) (Fig. 7.2).

CHANGES IN PRODUCTION

Changes in production were measured as changes in cover and density. Measurement of cover was carried out by the point quadrat method. A frame with thirteen pin holes set 3.8 cm apart was used to record plant presence in the quadrats. One hundred and eighty-two pin hits were recorded in each quadrat, giving a 3.8 cm x 3.0 cm resolution. As plant density was very low initially, and cover, owing to small individual plant sizes,

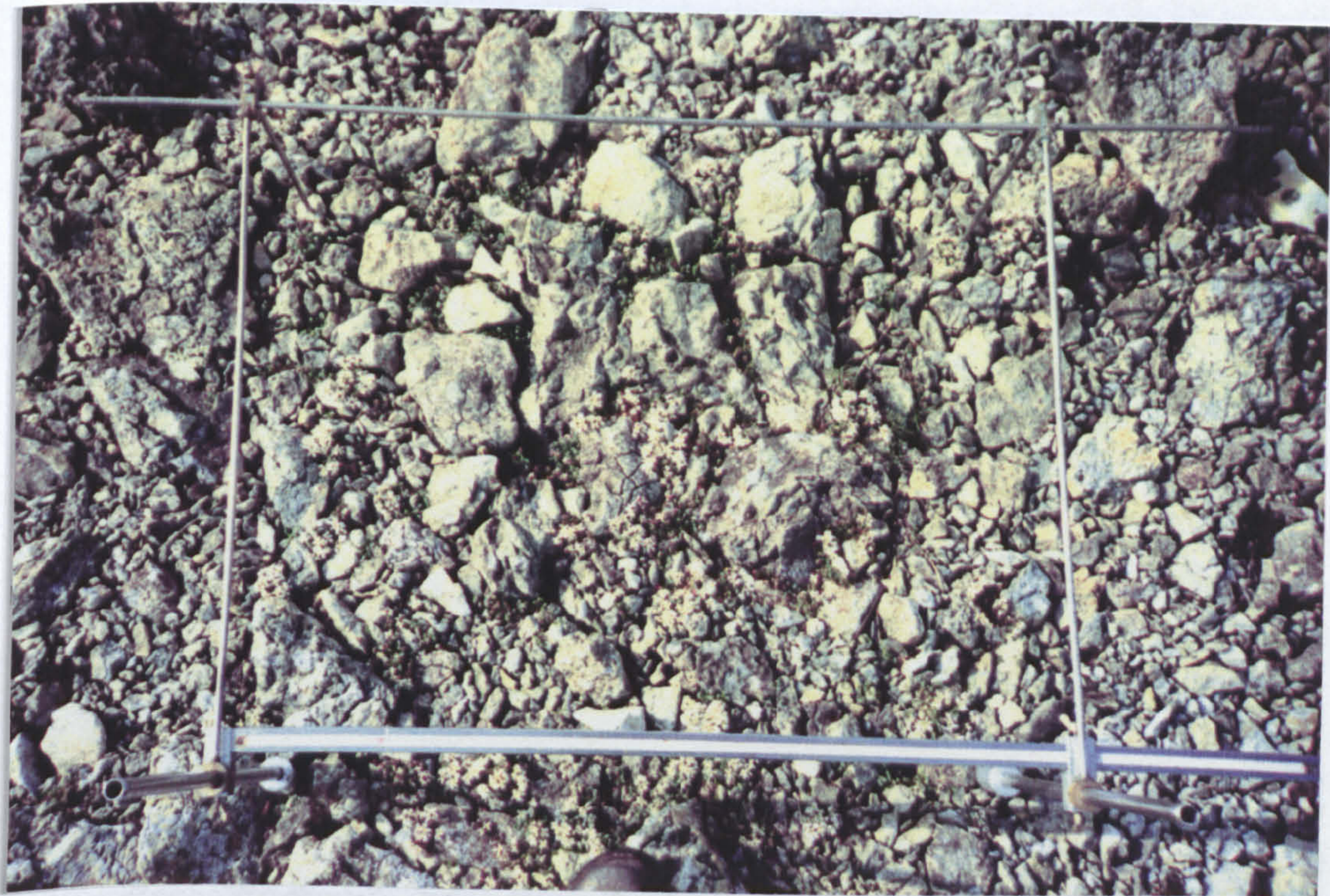


Fig 7.1 The metal frame used to record the position of individual plants. A perspex sheet (not shown) was placed on top of the frame to facilitate the recording.

...ity low (about 5%), no ...
... cover was estimated using ...

SUMMARY

To investigate the effects of ...
... were made at ...
... and the number of ...



Fig 7.2 MK1.5 with the enclosure cages over the experimental quadrats used for the nutrient addition experiment.

was very low (about 5%), no measure of cover was made until 29 May 1993. Before that date cover was estimated using the initial density values for each quadrat.

LIFE HISTORY

To investigate the effects of fertiliser addition on the development of individuals, size measurements were made on five randomly selected specimens in each quadrat. Rosette diameter and the number of rosette leaves were recorded for specimens of 12 months age. Plants were also assessed for the presence or absence of flower buds on 4 August 1993. Reproductive output was compared in the different treatments. Five specimens per quadrat were selected and the number of siliculae per individual counted on 25 July 1993.

SOIL AND PLANT CHEMICAL ANALYSES

Soil samples for chemical analysis were collected from each quadrat on 4 May 1993. For exchangeable cations 5 g of fresh soil was extracted by leaching four times with 25 ml 1M ammonium acetate. The bicarbonate method (Olsen) was used to extract the soils for available phosphorus (Allen 1989). The samples were also extracted with ion exchange resin bags for a comparative methodological study and the results are not reported here. Analytical methods followed those described in Chapter II.

Cochlearia plants were collected for elemental analysis from each quadrat in May 1993. The samples were digested in a concentrated sulphuric acid - hydrogen peroxide mix (Allen 1989) in a block digester at 330 °C. Atomic absorption spectrophotometry and determination of phosphorus were identical as described earlier (Chapter II).

CARBON ALLOCATION: TOTAL NON-STRUCTURAL CARBOHYDRATES

To assess seasonal changes in total non-structural carbohydrate (TNC) content in the different treatments, plants were collected from just outside the 50 cm x 42 cm quadrats from the buffer zones (the 15-cm strips outside the quadrats used for recording population dynamics which had also been fertilised to counter any edge effect) of each of the control, +Ca, +CaNPK, and +NPK treated quadrats on 2 May and 3 August 1993. The plants collected in May were kept in plastic bags on ice until transported to the laboratory within 12 h. After cleaning the plants with tap water they were immersed in liquid nitrogen to kill living tissue and then were kept in a freezer until further processing.

Soluble carbohydrates

Samples were separated into above-ground and below-ground parts and homogenised with acid-washed sand using a pestle and mortar. The samples were extracted in two changes of 80% ethanol at 60 °C for 1 h each. The extracts were centrifuged prior to decanting to prevent loss of starch. The phenol-sulphuric acid method was used to determine soluble sugar concentrations (Farrar 1993). For calibration, D-glucose standards were used. In August, samples were diced after separation into above-ground and below-ground parts and boiled in 80% ethanol for 1-3 h before transferring them to the laboratory. Samples were extracted at 70 °C for 1 h and then dried at 70 °C for 24 h to obtain the dry weights for the alcohol-insoluble residues. The dried samples were re-extracted after grinding. The redried samples were extracted for starch.

Starch

The residues after extraction for soluble carbohydrates were used to determine starch in the roots and shoots. The residues were enzymatically digested using amyloglucosidase (lyophilised powder from Aspergillus niger, Sigma) in 2 ml (5 units per 1 ml in May and 10 units per 1 ml in August) enzyme preparation at pH 4.5 with 0.2 mM MES buffer at 40 °C for 24 h. The sugar concentrations of the samples then were determined with the phenol-sulphuric method after centrifuging the digests.

CARBON ALLOCATION INTO ROOTS AND SHOOTS

The plants collected for TNC and chemical analyses were used to calculate root:shoot ratios. Fresh weights were recorded for roots and shoots, including rootstocks where applicable, after washing the roots in tap water and blotting off water from the surface. The samples then were dried at 90 °C for 3 h, then for a further 45 h at 70 °C.

STATISTICAL ANALYSES

One-way analyses of variance with the Tukey test were used to detect significant treatment effects on cover, change in population size, age structure, leafiness, average rosette size, reproductive output, proportion of flowering individuals and TNC. Data were arcsine-transformed, or log-transformed as appropriate before analysis.

Results

SOIL ANALYSES

The results for the soil analyses on samples collected from control and fertilised quadrats are given in Table 7.1. Phosphorus concentrations in the +NPK and +NPKCa were significantly higher than in the +Ca, however, they did not differ from the control. This was caused by the high variation in the values in the +Ca and the +NPKCa which caused high values for the pooled standard deviation in the ANOVA. Calcium was statistically higher in the +Ca and the +NPKCa where the concentrations of extractable Ca^{2+} doubled. As a result the Mg/Ca quotients were halved there. Although there was an increase in K^+ in the +NPK and +NPKCa it was not significant.

POPULATION DYNAMICS

Figures 7.3 and 7.4 shows the overall changes in population sizes of Cochlearia pyrenaica ssp. alpina in control, +Ca, +CaNPK and +NPK plots over the years 1991-1993. No major change of flux was observed in the control treatment (Fig. 7.3a,b), while the fertiliser treatments caused an increase in the number of recruitment, deaths and overall population sizes. The only significant change observed in 1992 following the fertiliser application in 1991 was in +Ca where percentage mortality was lower ($p = 0.05$) than in the other treatments. There was a dramatic increase in population sizes in +NPK and +NPKCa through an explosion in seedling recruitment in 1993 (Fig. 7.4a,b). The annual rates of increase in population sizes ($\lambda = N_t/N_{t-1}$) were 0.98 (control),

	pH	P (mg kg ⁻¹)	K (meq kg ⁻¹)	Ca (meq kg ⁻¹)	Mg (meq kg ⁻¹)	Ni (mg kg ⁻¹)	Mg/Ca
control	5.4	8.1a	0.9	11.9a	137.7	39.7	12.3a
+Ca	5.6	5.9ab	1.1	25.4b	140.6	37.3	5.6b
+NPK	5.6	30.2ac	1.8	9.6a	135.1	39.7	14.0a
+NPKCa	5.5	34.5ac	1.4	25.6b	132.9	39.3	5.2b

Table 7.1 Results of chemical analyses for soil samples collected in control, +Ca, +NPK and +NPKCa treated quadrats in May 1993. Values are means (n=3); identical letters indicate non significant differences (p=0.05) for individual elements.

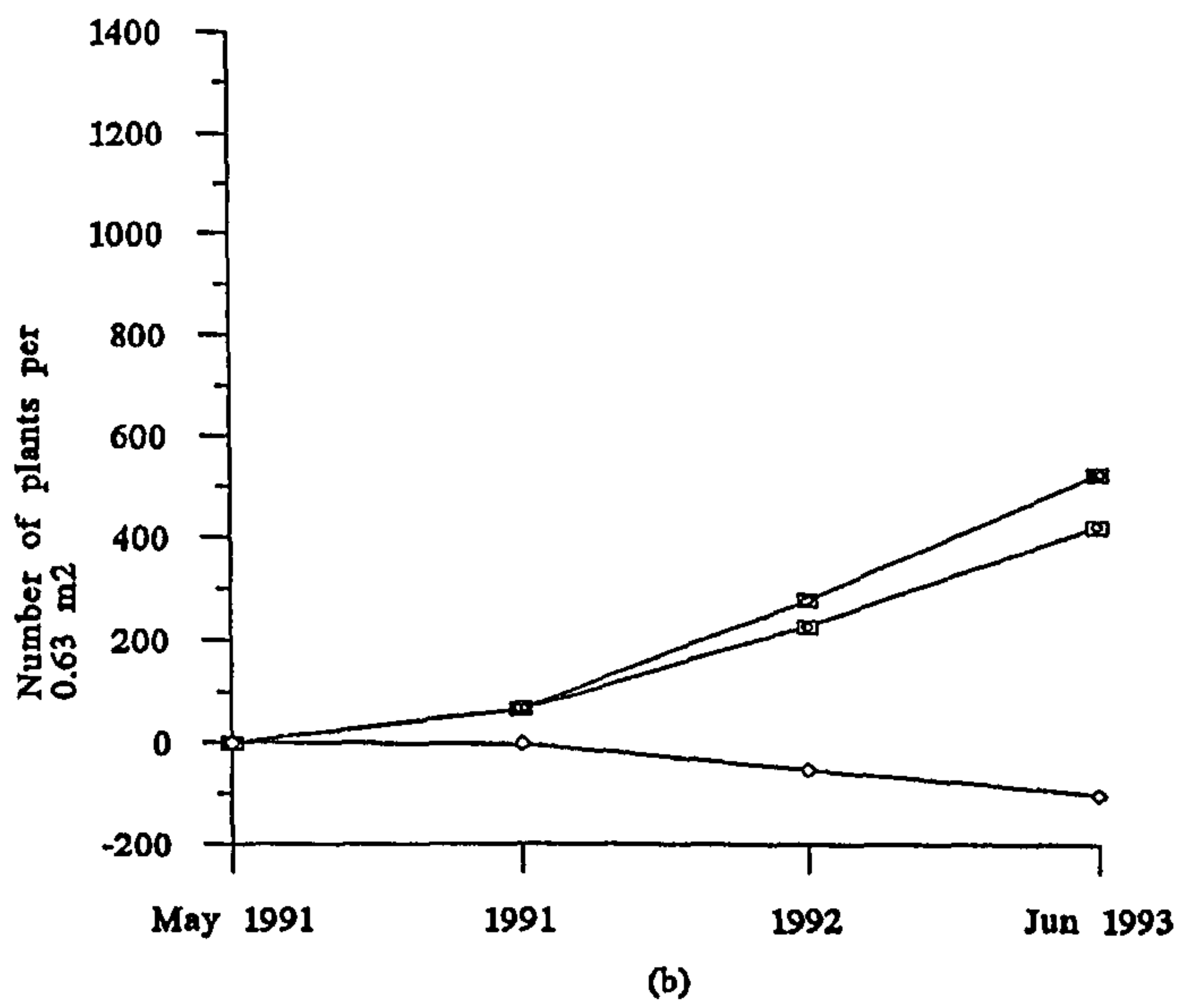
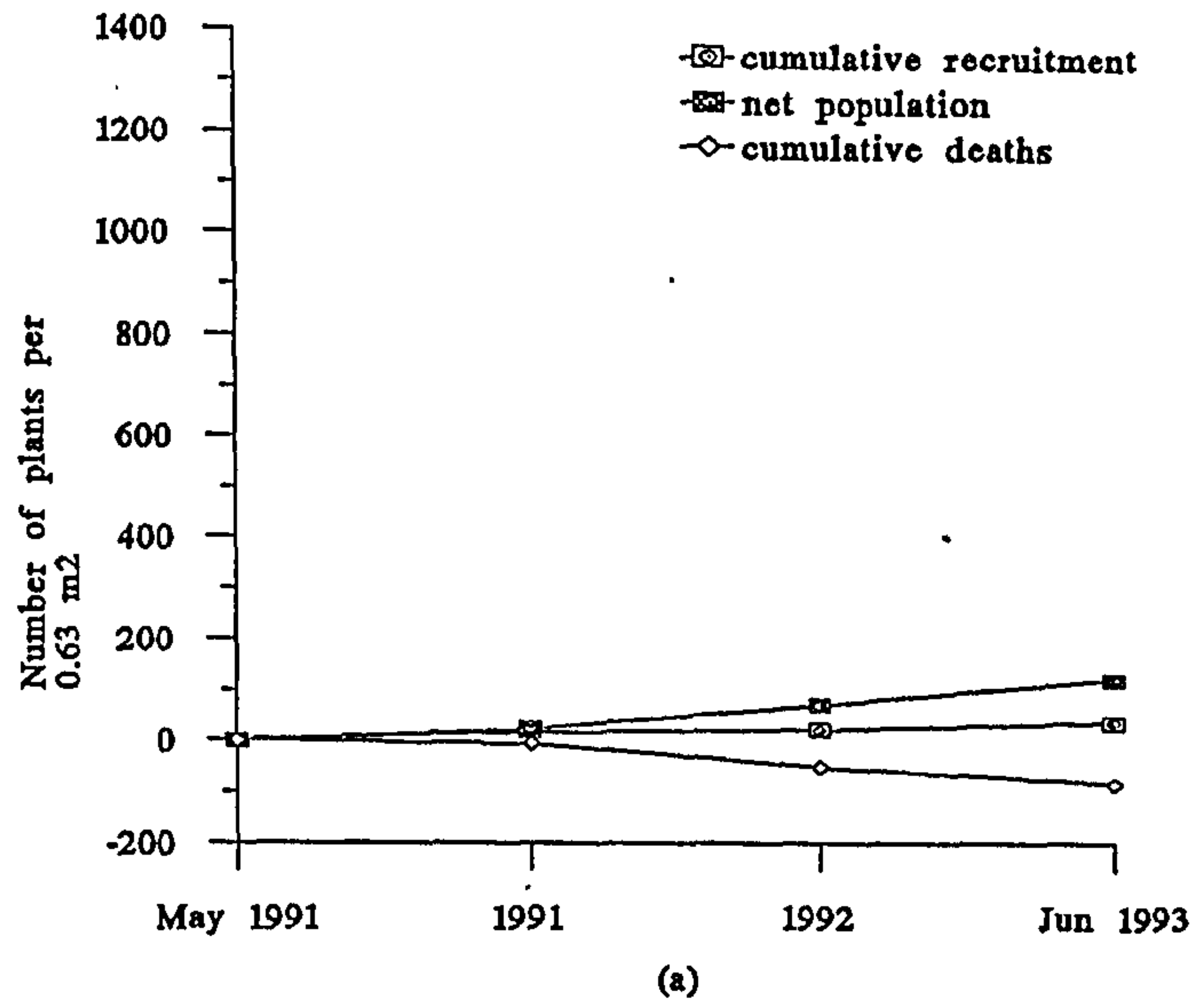


Fig 7.3 The flux of individuals of *Cochlearia pyrenaica* ssp. *alpina* in control (a) and +Ca (b) treatment at MK1.5 in 1991-1993.

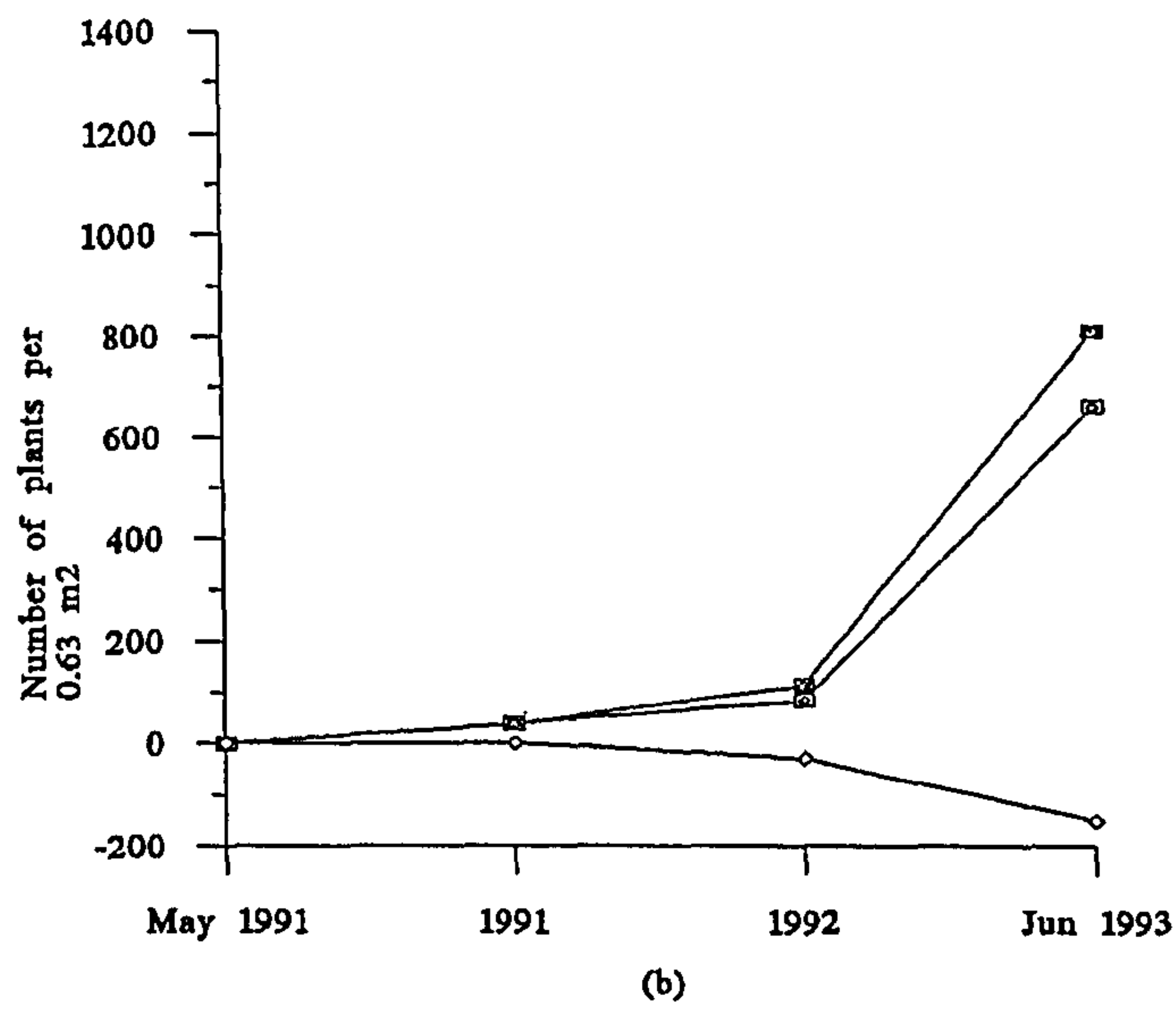
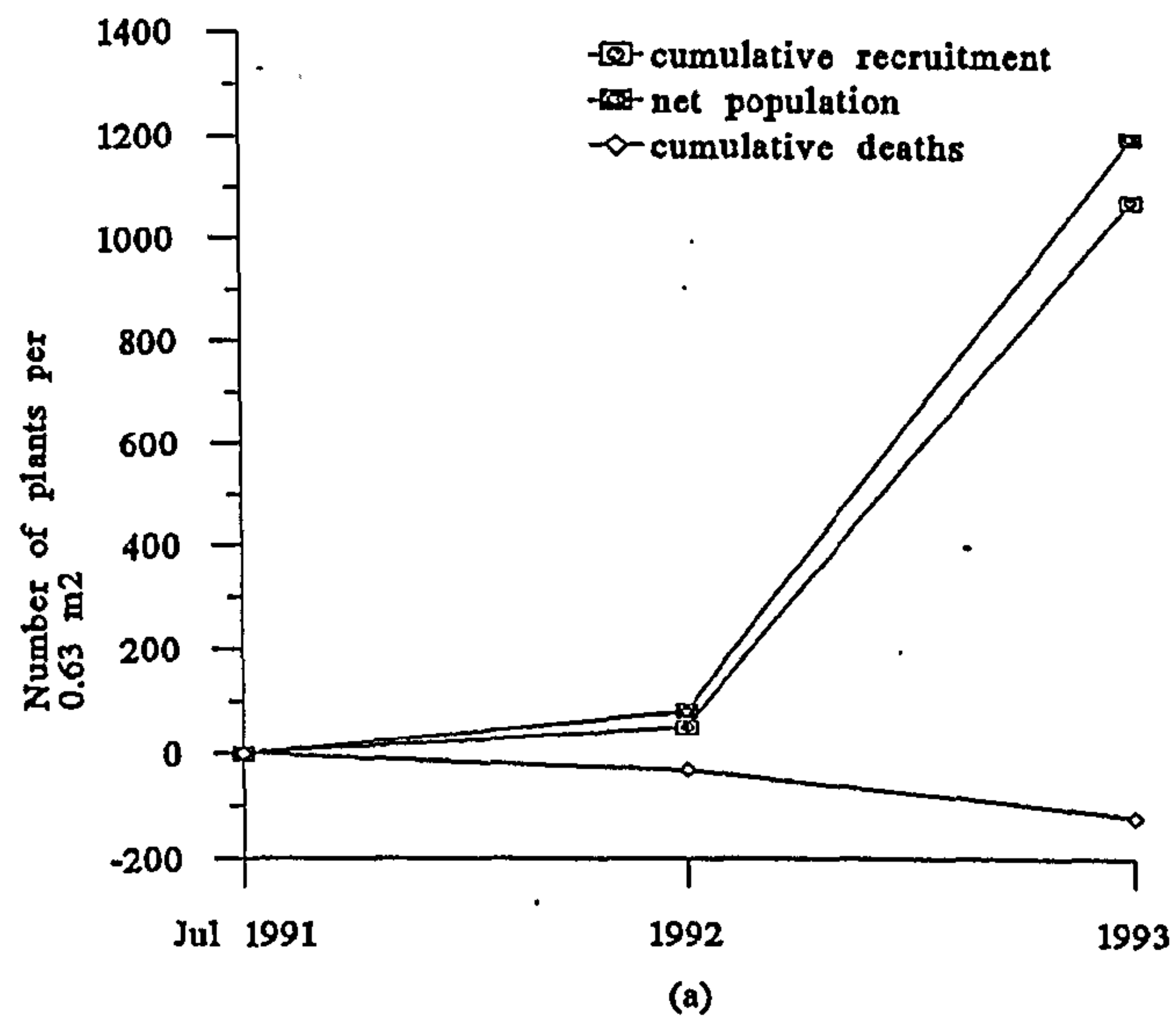


Fig 7.4 The flux of individuals of *Cochlearia pyrenaica* ssp. *alpina* in +NPK (a) and +NPKCa (b) treatments at MK1.5 in 1991-1993.

1.69 (+Ca), 1.38 (+NPK) and 1.19 (+NPKCa) for 1991-92. These values remained similar in the control (0.97) and the +Ca (1.51) and largely increased in the +NPK (5.82) and +NPKCa (4.60) for the 1992-93 period (Fig. 7.5).

The survivorship curves for the pre-treatment cohorts recorded in July 1991 are shown in Fig. 7.6. The control and +Ca-treated populations showed a constant depletion with no change in its rate over time. The populations growing in +NPK and +NPKCa, however showed a marked change in the rate of depletion after 1992. The initial gentle slope of mortality, which did not differ from those of the control and +Ca, became very steep after 1992 indicating high mortality.

The age structure, defined as the number of adults (> 1 yr old) versus seedlings is shown for the populations under different treatments in Fig. 7.7. There was no significant change in the control population (Fig. 7.7a). The proportion of seedlings after calcium addition increased from the initial 19.3% to 43.5% in 1992 and did not change thereafter (Fig. 7.7b). The age structure in +NPK (Fig. 7.7c) and +NPKCa (Fig. 7.7d) remained very similar to the original, one year after the fertiliser addition. This was followed however by a very high increase in seedling numbers with the absolute numbers of the adults largely unchanged.

About 5-6% of the adult (> 1 yr old) plants flowered in the control and +Ca in all three years and in the +NPK and +NPKCa before fertilisation (Fig. 7.8). +NPK and +NPKCa increased the proportion of flowering adults by about eleven- and eight-fold in 1992 and by about five- and six-fold in 1993.

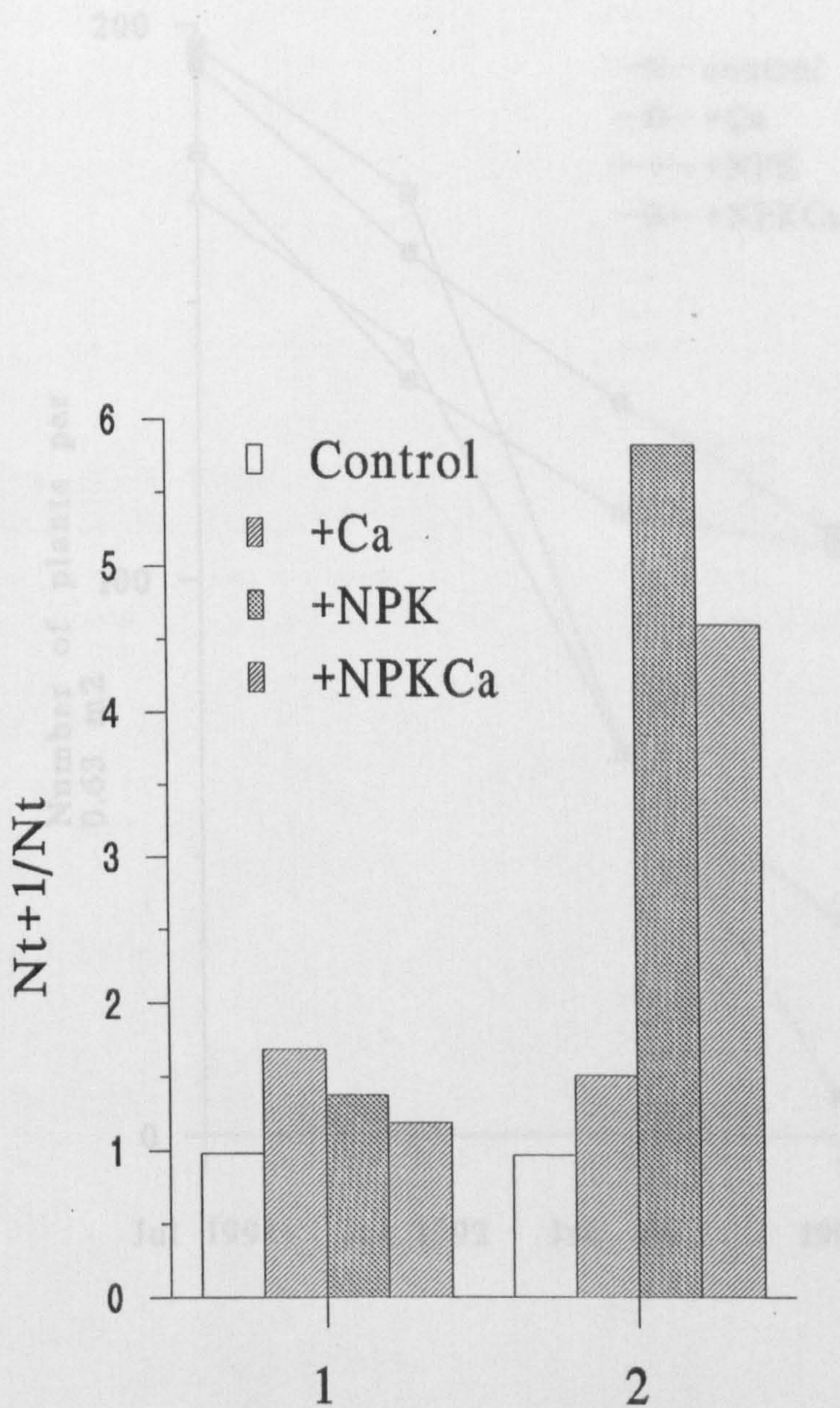


Fig 7.6 Depletion curves for the original (1991) cohorts of *Cochlearia pyrenaica* ssp. *alpina* in control, +Ca, +NPK and +NPKCa treatments. Values for 1994 were estimated based on the numbers of flowering specimens in 1993.

Fig 7.5 The annual rate of increase ($\lambda=N_t/N_{t-1}$) of the *Cochlearia pyrenaica* ssp. *alpina* populations in various fertiliser treatments in 1991-92 (1) and 1992-93 (2)

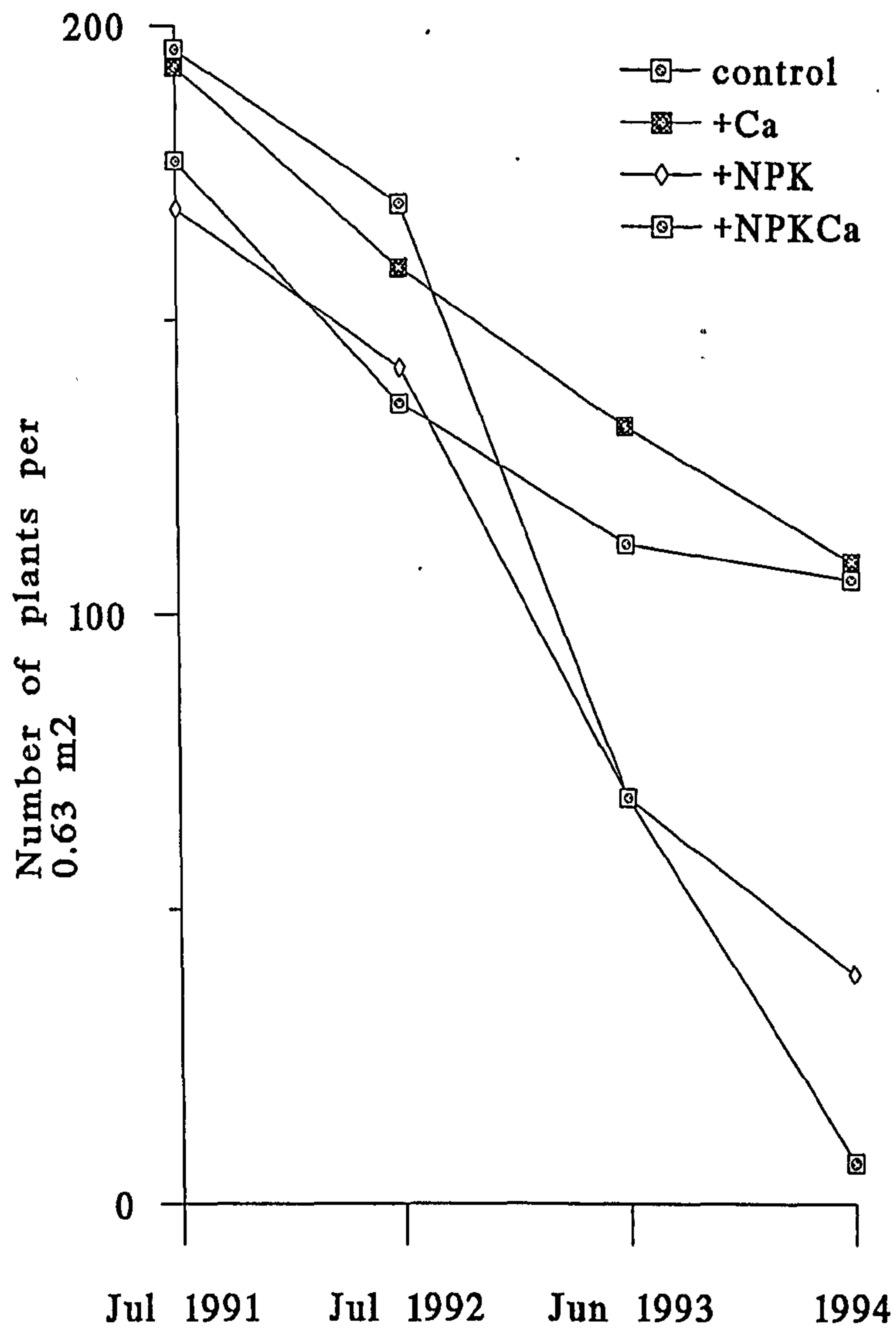


Fig 7.6 Depletion curves for the original (1991) cohorts of *Cochlearia pyrenaica* ssp. *alpina* in control, +Ca, +NPK and +NPKCa treatments. Values for 1994 are estimates based on the numbers of flowering specimens in 1993.

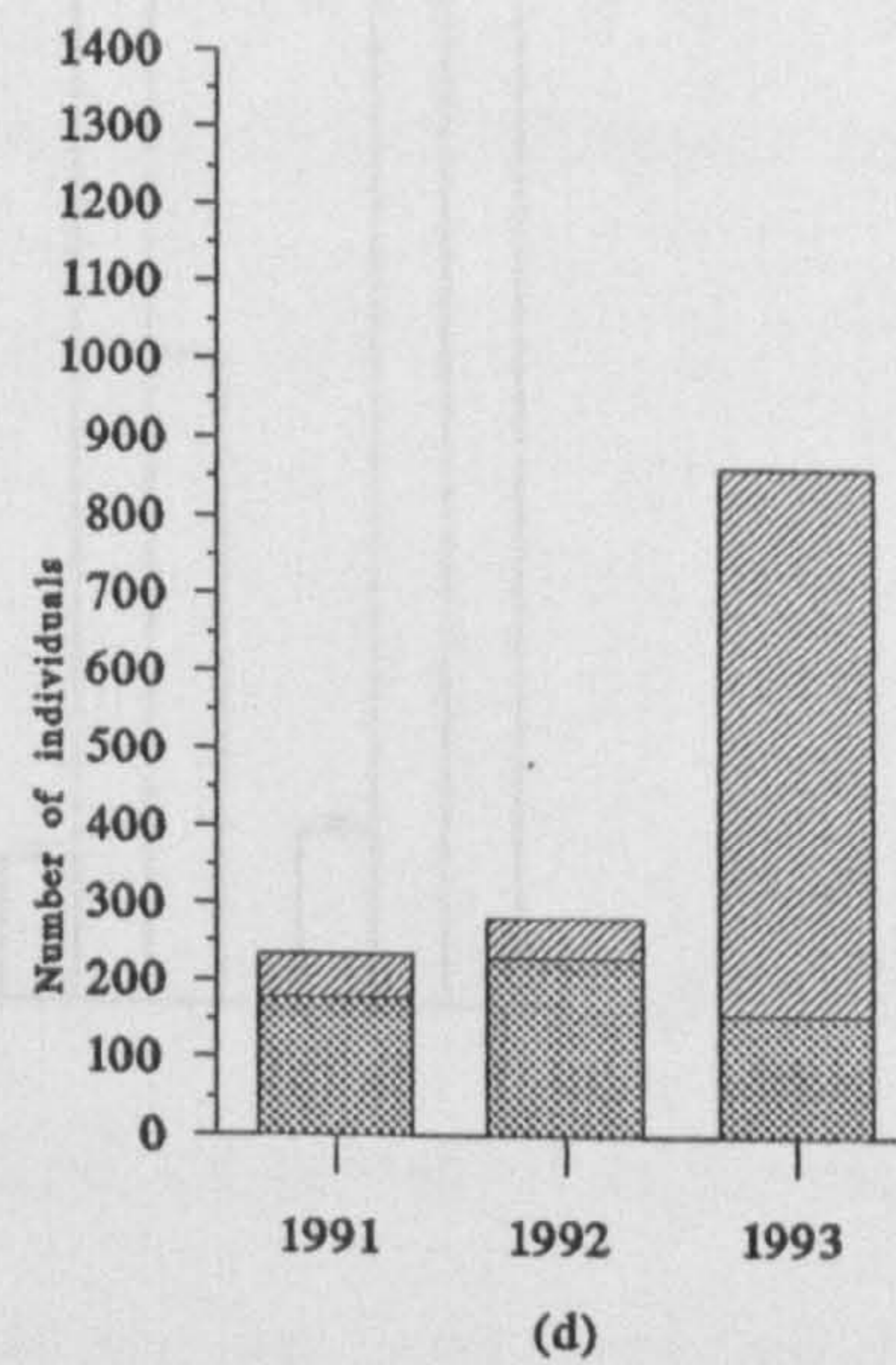
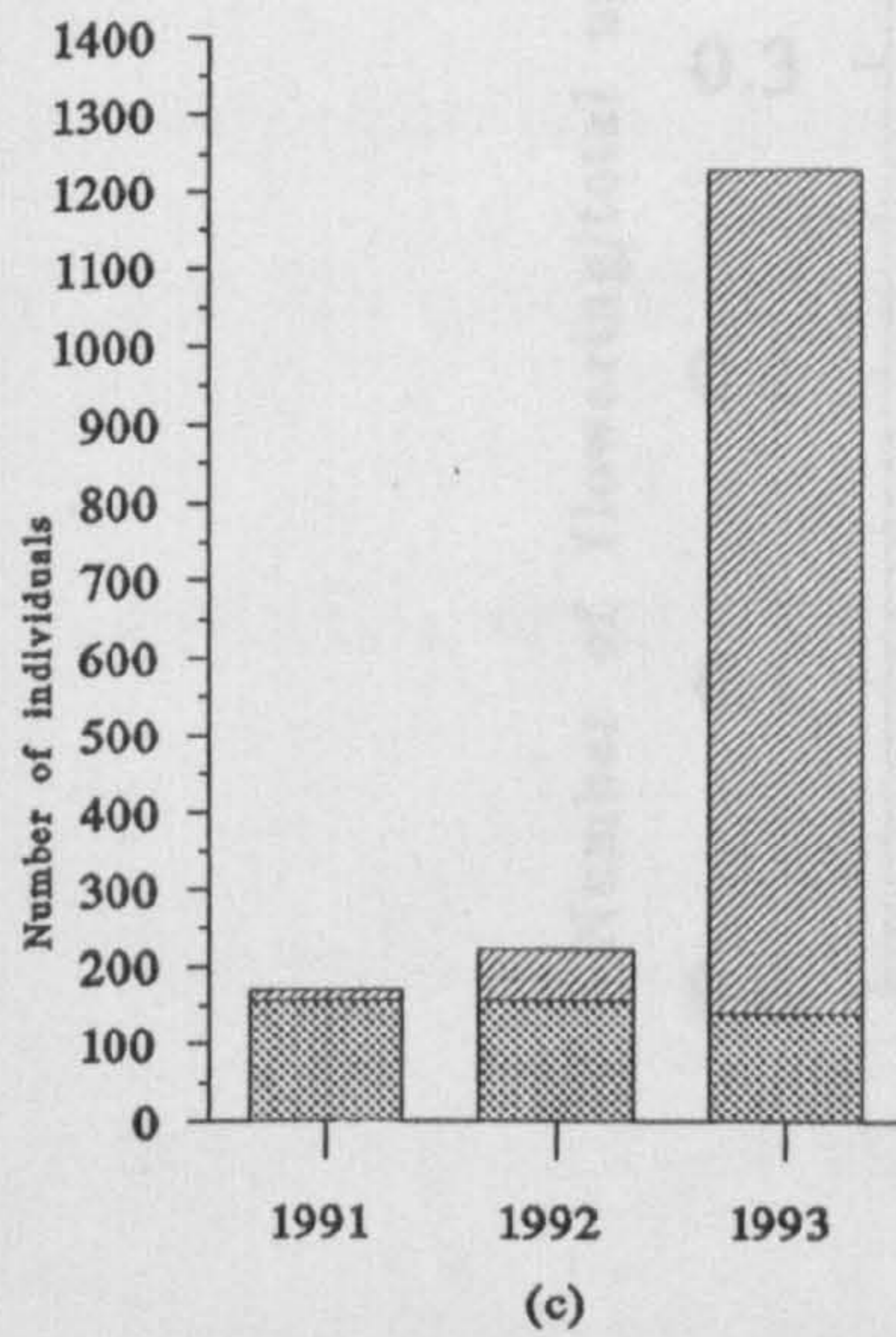
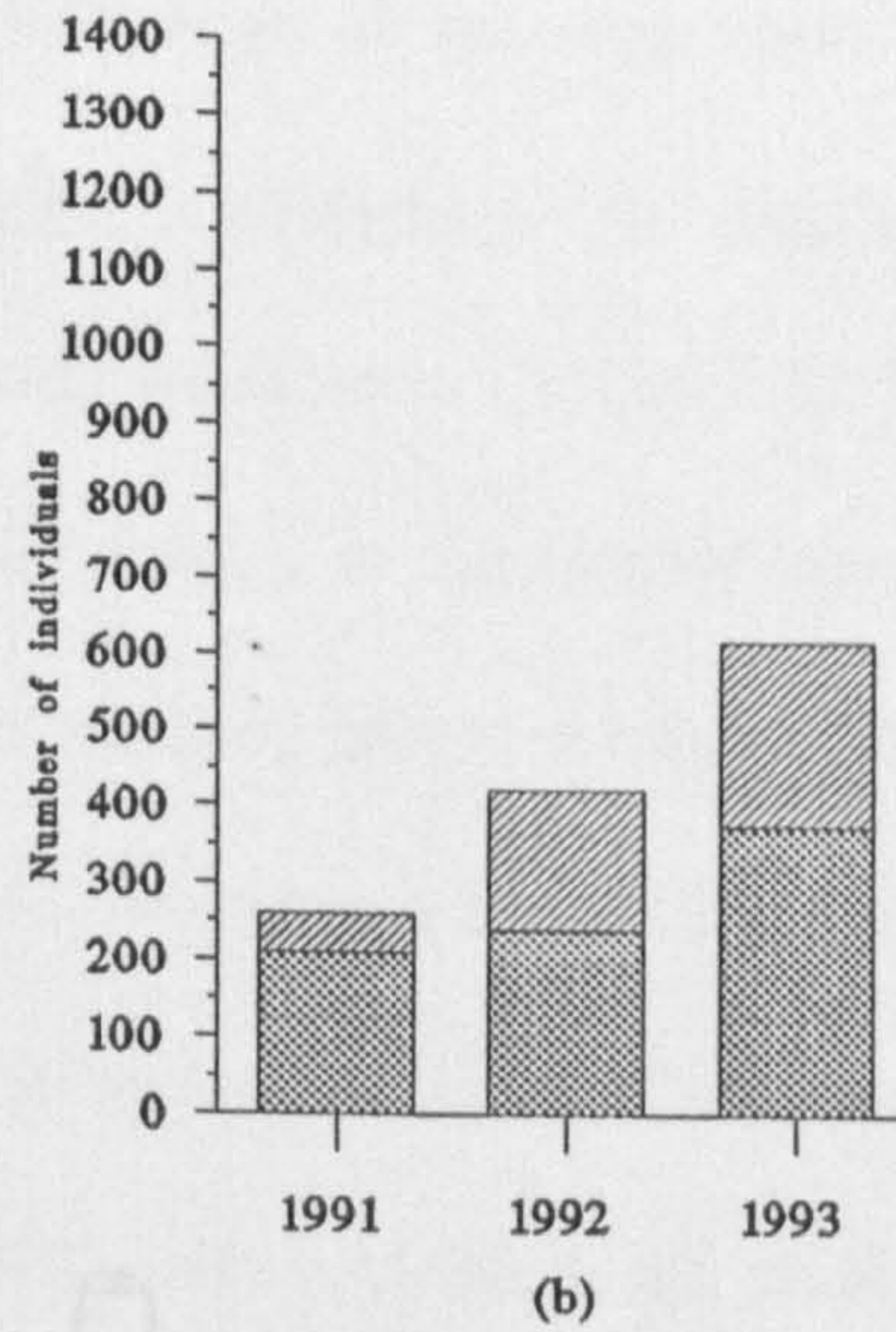
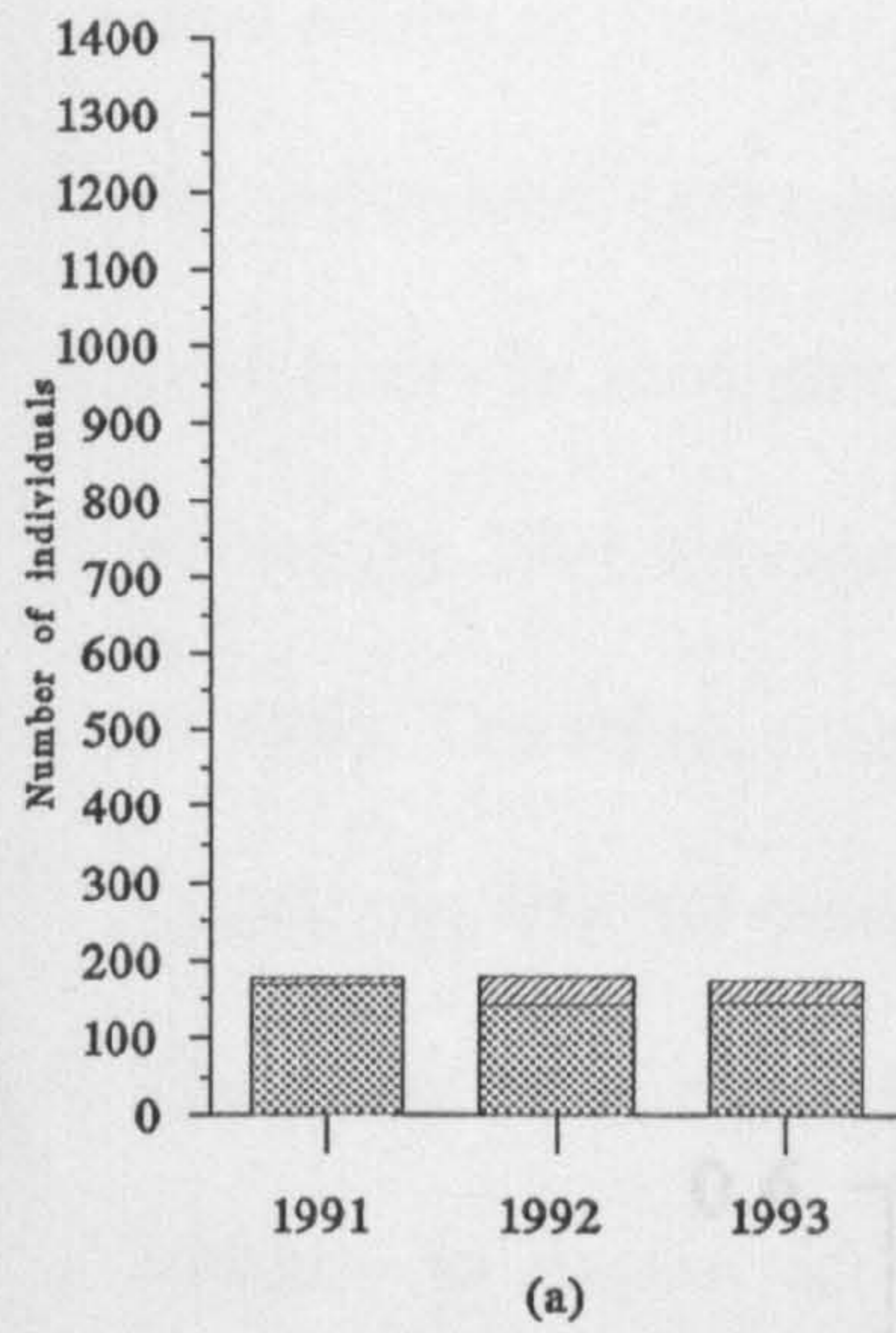


Fig 7.7 The age structure of the populations of *Cochlearia pyrenaica* ssp. *alpina* in control (a), +Ca (b), +NPK (c) and +NPKCa (d) treatments. Lower portion of bars represent plants of > 1 y age and upper portion of bars show the number of seedlings.

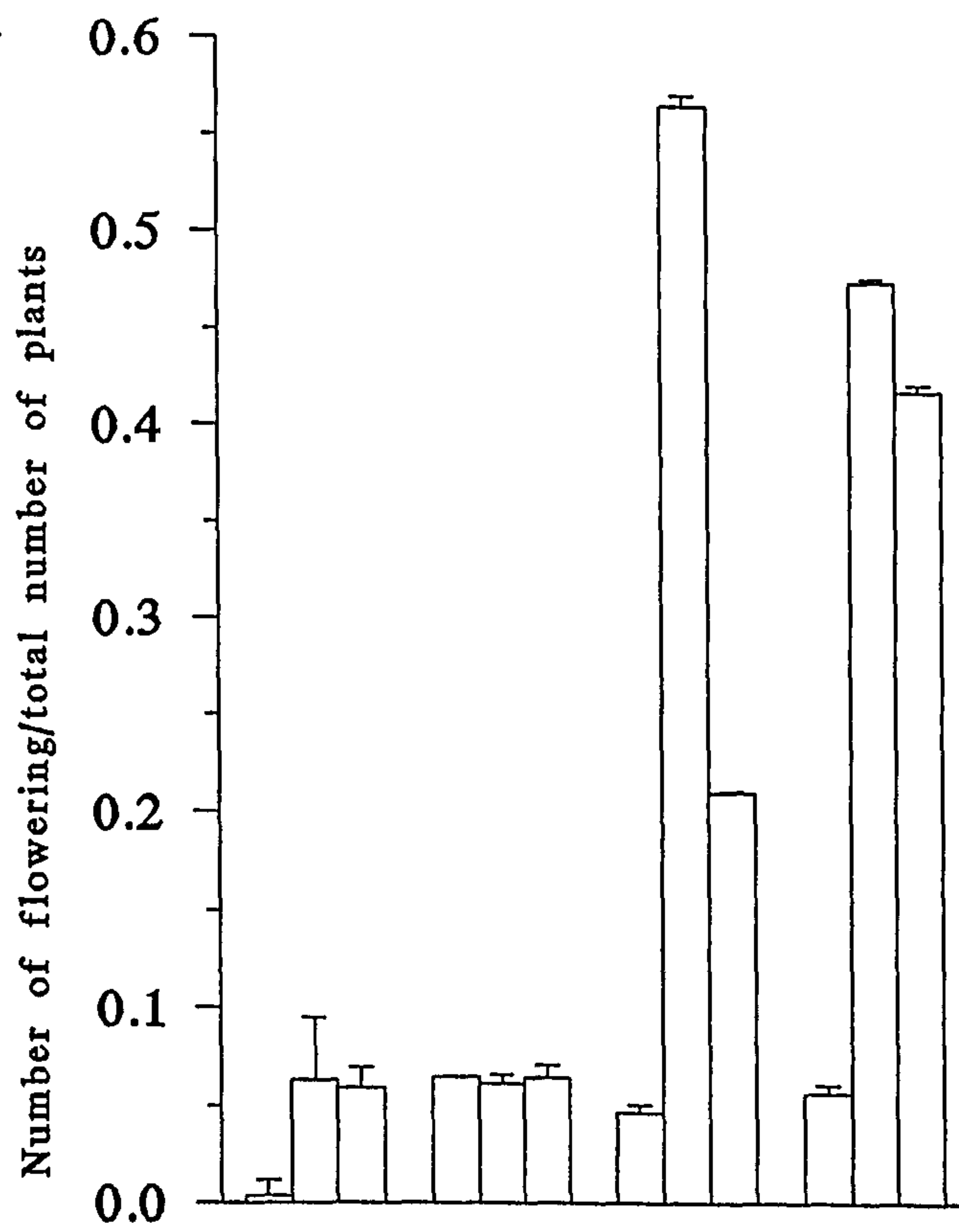


Fig 7.8 The proportion of flowering specimens to the total number of adult plants (age > 1 y) in *Cochlearia pyrenaica* ssp. *alpina* in control (1), +Ca (2), +NPK (3) and +NPKCa (4) in 1991, 1992 and 1993. Bars show s.e.

PRODUCTION

The effects of fertiliser application on the production of the vegetation were assessed by differences between the cover of Cochlearia pyrenaica ssp. alpina and also by differences in total plant cover in the different treatments (Table 7.2). The estimated values for 1991 are calculated from cover and density in the control quadrats recorded in 1993. The initial dramatic increase on the addition of the fertilisers in the cover of Cochlearia was not quantified in October 1991, however it is well illustrated in Fig 7.9. The cover by Cochlearia was between 3.3% and 4.2% in 1991 before the fertiliser addition in August of that year. +Ca, +NPK, and +NPKCa all caused significant increases in percentage cover by 1993 (Table 7.2a). +NPKCa was significantly higher than +Ca and +NPK. There were also significant differences in total cover in 1993. The original total cover was 5.3-6.6% (Table 7.2b). In 1993, the control and +Ca did not differ significantly, while +NPK and +NPKCa significantly increased cover in comparison with the control and +Ca, with no significant differences between +NPK and +Ca ($p = 0.05$) (Table 7.2b).

CHEMICAL ANALYSES OF PLANT TISSUE

The concentrations of nitrogen, phosphorus, potassium, calcium, magnesium and nickel in above-ground and below-ground plant tissue are presented in Tables 7.3 and 7.4. Shoot nitrogen was significantly higher in all addition treatments than in the control samples (Table 7.3). Phosphorus concentrations were increased in all treatments, with +NPK and +NPKCa plants having significantly higher phosphorus concentrations than

	1991	1993
Control (% cover)	3.8	3.7
+Ca (% cover)	4.2	13.9 a
+NPK (% cover)	3.3	20.3 a
+NPKCa (% cover)	3.6	28.4

(a)

	1991	1993
Control (% cover)	6.4	6.6 a
+Ca (% cover)	6.6	23.1 a,c
+NPK (% cover)	5.3	38.7 b,c
+NPKCa (% cover)	5.4	59.3 b

(b)

Table 7.2 Average percentage cover of *Cochlearia pyrenaica* subsp. *alpina* (a) and of all species (b) in control, +Ca, +NPK and +NPKCa quadrats in 1991 and in 1993 following two year's fertiliser application. Values are means (n=3). Differences between treatments with identical letters are not significant at p=0.05. Values for 1991 are estimates based on density and cover values in control in 1993.



(a)



(b)

Fig 7.9 *Cochlearia pyrenaica* ssp. *alpina* in a +NPKCa fertilised quadrat (a) and in the control (b) in October 1991.

	Nitrogen ($\mu\text{g g}^{-1}$ d.w.)	Phosphorus ($\mu\text{g g}^{-1}$ d.w.)	Potassium ($\mu\text{g g}^{-1}$ d.w.)	Calcium ($\mu\text{g g}^{-1}$ d.w.)	Magnesium ($\mu\text{g g}^{-1}$ d.w.)	Nickel ($\mu\text{g g}^{-1}$ d.w.)
Control (n=7)	36700a	2200a	17800a	1860a	8920	299
+Ca (n=9)	47300b	3920b	21800ab	2980b	11200	439
+NPK (n=8)	53400b	5730c	22700ab	1710a	10600	156
+NPKCa (n=10)	49100b	6050c	26000b	3920b	9410	188

Table 7.3 Mean concentrations ($\mu\text{g g}^{-1}$ d.w.) of nitrogen, phosphorus, potassium, calcium, magnesium and nickel in the shoots of Cochlearia pyrenaica ssp. alpina in control, +Ca, +NPK and +NPKCa treatments in May 1993. There were no significant differences in magnesium and nickel between the different treatments; for the other elements identical letters indicate no differences in their concentrations between treatments ($p \leq 0.05$).

	Nitrogen ($\mu\text{g g}^{-1}$ d.w.)	Phosphorus ($\mu\text{g g}^{-1}$ d.w.)	Potassium ($\mu\text{g g}^{-1}$ d.w.)	Calcium ($\mu\text{g g}^{-1}$ d.w.)	Magnesium ($\mu\text{g g}^{-1}$ d.w.)	Nickel ($\mu\text{g g}^{-1}$ d.w.)
Control (n=7)	34900	1250a	9860a	2590a	8010	434ab
+Ca (n=9)	36900	3670a	13300ab	3330a	9880	598b
+NPK (n=8)	38700	6580b	11800ab	2800a	12300	351a
+NPKCa (n=10)	36500	7970b	14900b	4980b	10600	417ab

Table 7.4 The concentrations ($\mu\text{g g}^{-1}$ d.w.) of nitrogen, phosphorus, potassium, calcium, magnesium and nickel in control, +Ca, +NPK and +NPKCa treatments in the roots of *Cochlearia pyrenaica* ssp. *alpina* in May 1993. For phosphorus, potassium, calcium ($p \leq 0.05$) and nickel ($p \leq 0.06$) significant differences between treatments are indicated by different suffixes. No significant differences were found for nitrogen and magnesium.

+Ca plants. Calcium concentrations were significantly higher in +Ca and +NPKCa than in control or +NPK with no difference between +Ca and +NPKCa. Nickel concentrations were not statistically different between treatments, though slightly reduced in the shoots in +NPK and +NPKCa.

Root nitrogen concentrations did not differ in the different treatments (Table 7.4). Phosphorus addition resulted in significantly higher concentrations in the roots and +Ca also caused higher tissue phosphorus. No significant differences were found in calcium concentrations between treatments with the exception of +NPKCa. The magnesium concentrations in both the roots and shoots were similar in all the treatments. Nickel concentrations were lower in +NPK than in +Ca, where they were highest ($p=0.06$).

LIFE HISTORY

Average rosette sizes and number of leaves per plant are shown in Figure 7.10. There was a three-fold increase in average rosette diameters in +NPK and +NPKCa compared with the control and their difference from +Ca was also significant ($p=0.001$) (Fig. 7.10a). Leaf numbers were significantly higher in +NPK and +NPKCa (Fig. 7.10b). There was also a significant difference ($p=0.01$) between the number of leaves produced by +NPK and +NPKCa plants, the former having twenty-seven on average against the seventeen of the latter.

The average number of siliculae per plant produced in the different treatments is shown in Fig. 7.11. The numbers of siliculae increased on the addition of fertilisers in the order +Ca > +NPK > +NPKCa. Significant differences were observed between control and +NPK and +NPKCa, and also between +Ca and +NPK ($p=0.001$).

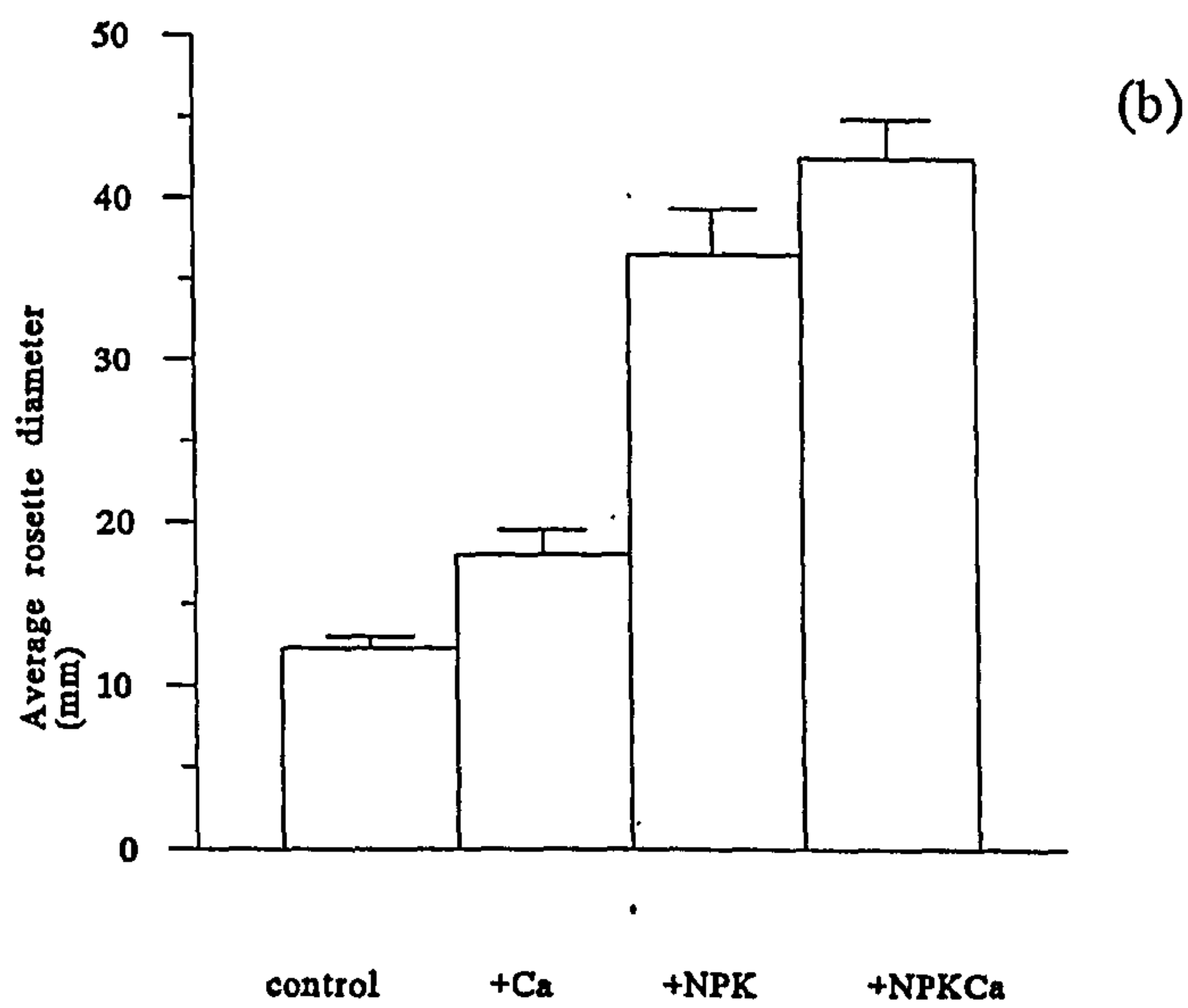
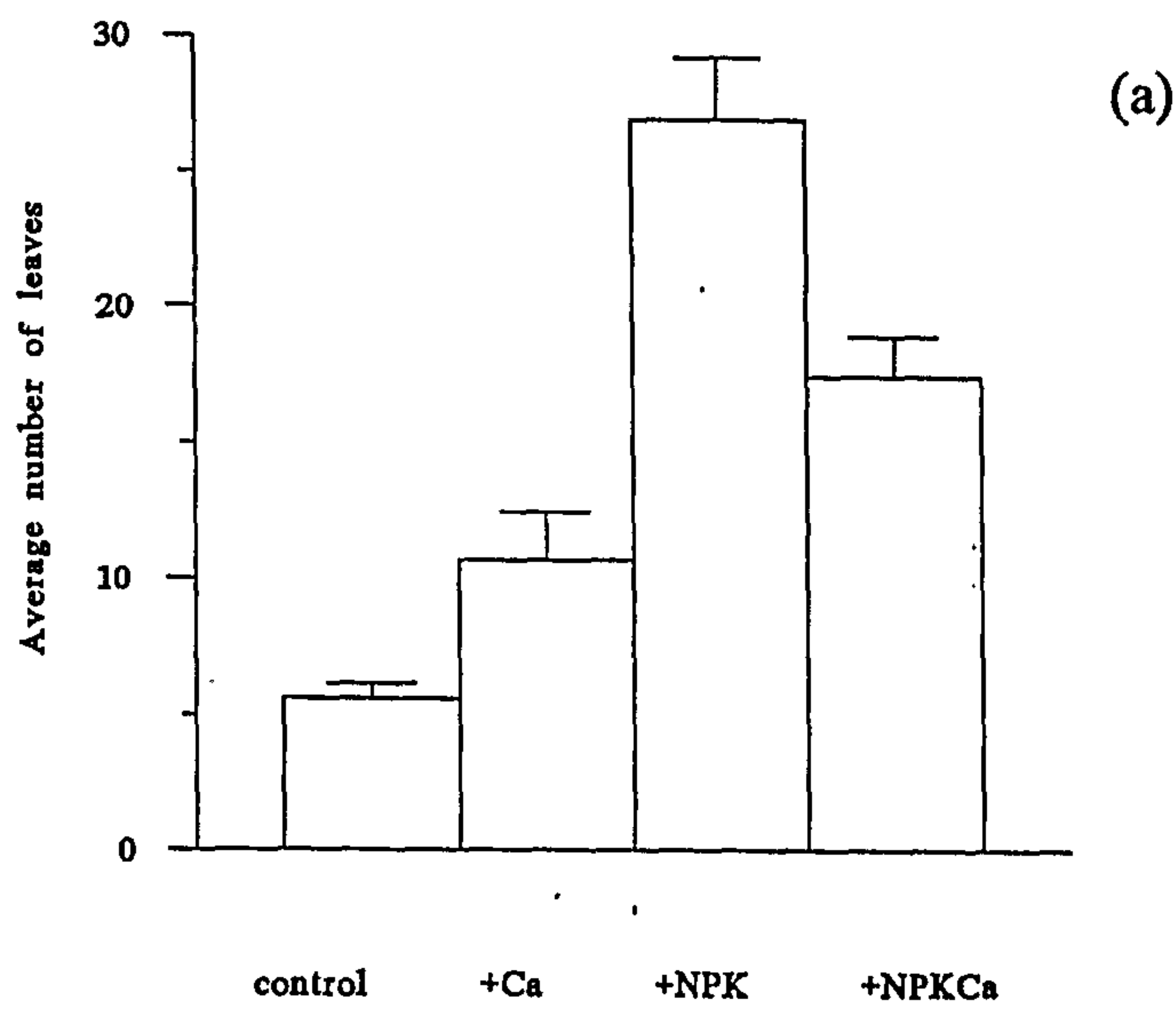


Fig 7.10 Average number of leaves per rosette (a) and average rosette diameter (b) in *Cochlearia pyrenaica* ssp. *alpina* in control, +Ca, +NPK and +NPKCa treatments at MK1.5 in 1993. Values are means (n=15) with s.e.

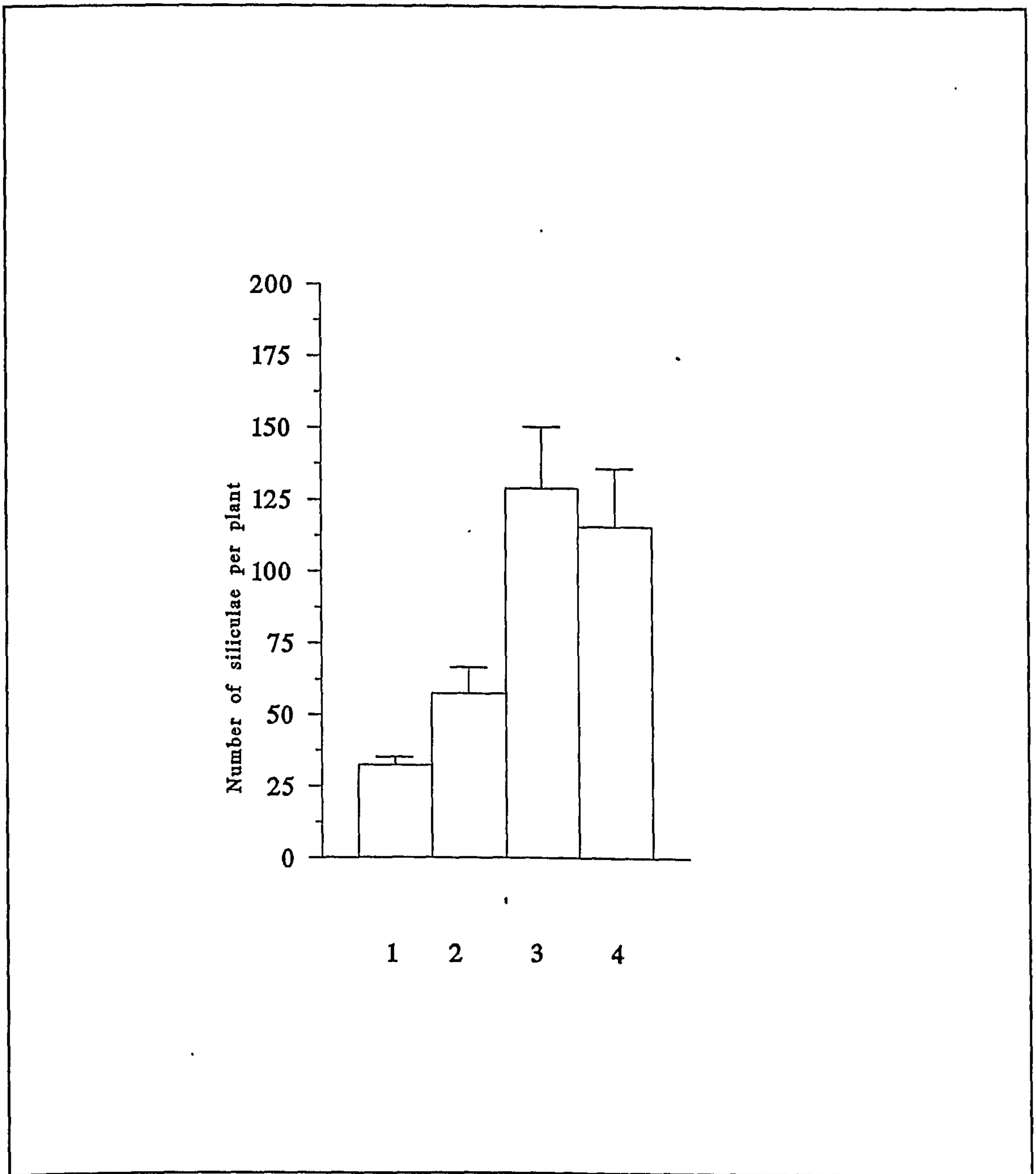


Fig 7.11 The mean number of siliculae produced by Cochlearia pyrenaica ssp. alpina in control (1), +Ca (2), +NPK (3) and +NPKCa (4) treatments in 1993. Values are means (n=9) with vertical bars showing s.e.

The results for the small-scale experiment set up in June 1993 showed that plants responded only in the +P treatment when they showed a change in colour and increase in size. Also, it was only in the +P treatment that plants produced flower buds.

CARBON ALLOCATION

Shoot:root ratio

Fresh-weight root:shoot ratios based on the samples collected in May, most of which were bearing flower buds, are shown in Table 7.5. Plants in control and +Ca allocated a higher proportion of assimilates to roots (mean root:shoot ratio \pm s.e., 0.68 ± 0.11 and 0.71 ± 0.12) than +NPK and +NPKCa (0.43 ± 0.04 and 0.42 ± 0.05).

The August samples, assumed to be recruited in 1992, did not conform to this pattern and the results showed no clear differences in carbon partitioning between above-ground and below-ground organs between the different treatments.

TNC allocation

Figure 7.12 shows the pattern of TNC allocation in Cochlearia pyrenaica ssp. alpina in May and August 1993. Mean TNC concentrations for roots and shoots showed no statistically significant differences between treatments on either sampling date. There was however a trend of decrease in TNC in the shoots in May and an increase in August in the order of control > +Ca > +NPK > +NPKCa. The August shoot samples contained higher TNC concentrations: 2.0 (control), 2.8 (+Ca), 4.3 (+NPK), and 4.7-fold

	May 1993	August 1993 (1 y)
Control (n=11; 3)	0.68 ±0.11	0.93 ±0.13
+Ca (n=12; 3)	0.71 ±0.12	0.57 ±0.01
+NPK (n=11; 3)	0.43 ±0.04	1.10 ±0.15
+NPKCa(n=13; 3)	0.42 ±0.05	0.63 ±0.17

Table 7.5 Average root:shoot ratio \pm s.e. in Cochlearia pyrenaica subsp. alpina in different fertiliser treatments in May and August 1993. May values are means of randomly collected specimens while August values are for specimens of 12 months age. The first number in parentheses for 'n' is for May and the second is for August

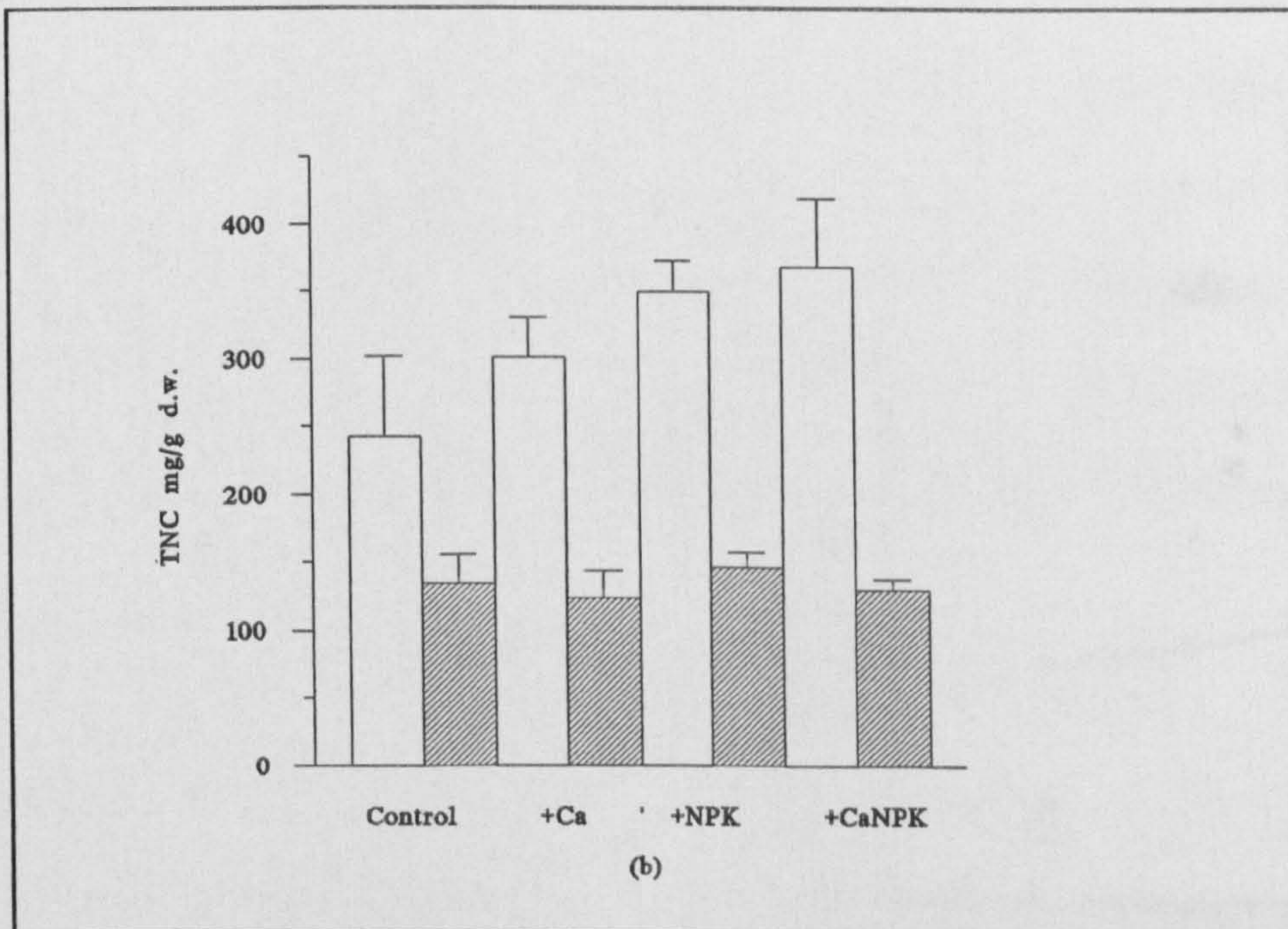
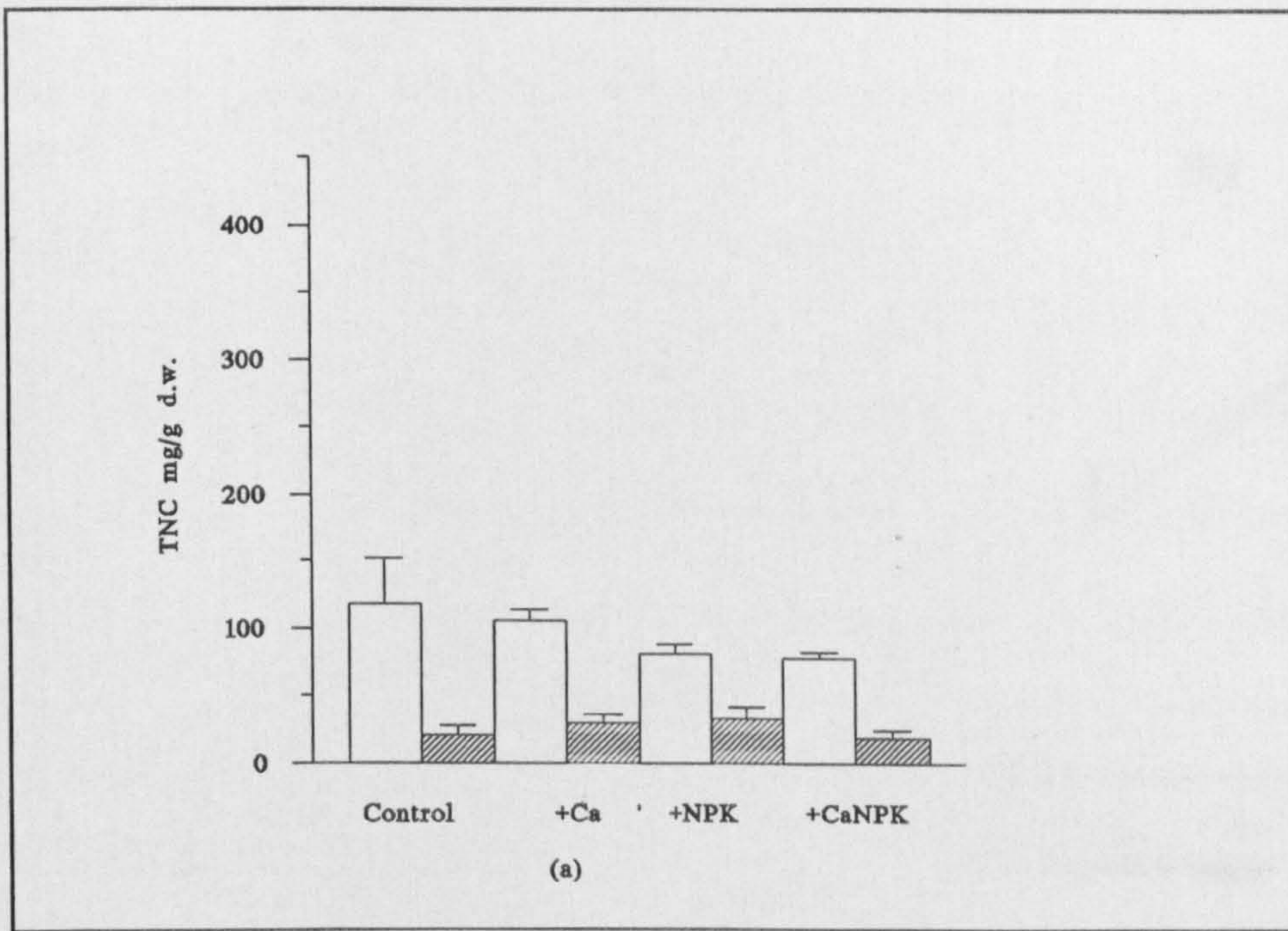


Fig 1.12 The concentration of total non-structural carbohydrates (TNC) in the shoots (unfilled bars) and in the roots (cross-hatched bars) of *Cochlearia pyrenaica* ssp. *alpina* in various fertiliser treatments in May (a) and in August (b) 1993. Values are means (n=3) with s.e.

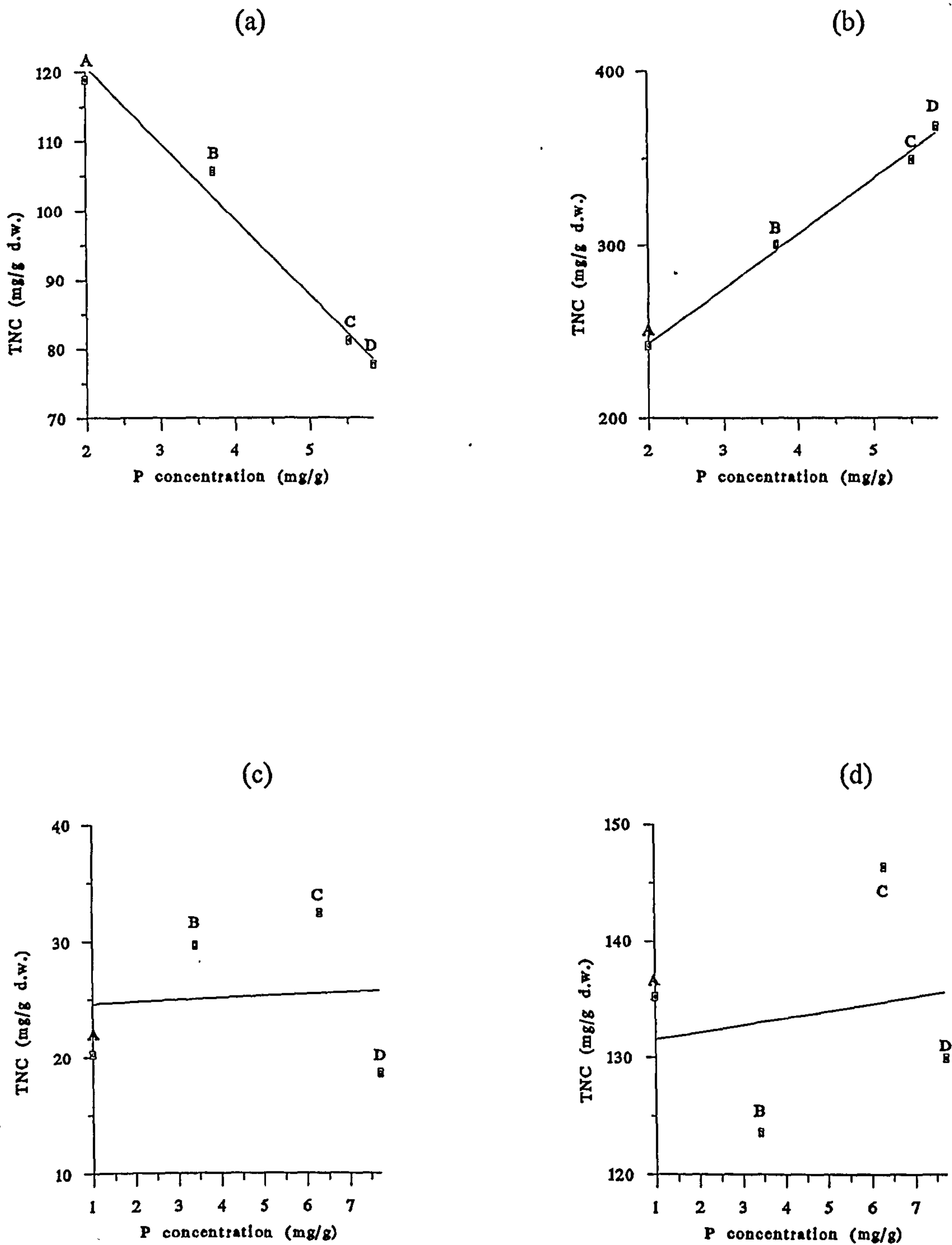


Fig 7.13 The relationship between shoot phosphorus and TNC concentrations in May (a) and August (b) 1993 and root phosphorus and TNC in May (c) and August (d) in *Cochlearia pyrenaica* ssp. *alpina* in control (A), +Ca (B), +NPK (C) and +NPKCa (D) treatments. The equations for the curves fitted by Cricket Graph were: (a) $y = 145 - 11x$, $r^2 = 0.98$; (b) $y = 180 - 92x$, $r^2 = 0.99$; (c) $y = 24 + 0.2x$, $r^2 = 0.00$; (d) $y = 191 + 0.6x$, $r^2 = 0.04$.

(+NPKCa) and between 4.2 and 7.0-fold in the roots with no apparent trend. For starch, the values were 2.5 (control), 3.9 (+Ca), 7.7 (+NPK), and 8.6 times (+NPKCa) higher in the shoots in August and between 2.9 and 10 times higher in the roots. The shoot starch:root starch ratios in May were 9.2 (control), 2.7 (+Ca), 3.1 (+NPK), and 5.4 (+NPKCa); values for August were between 4.3 and 4.6 in all treatments.

There was a linear relationship between shoot phosphorus concentration and shoot TNC (Fig. 7.13). The May samples contained less TNC with increasing phosphorus, while an inverse relationship was found in the August samples where plants contained increasingly more TNC in the different treatments with increased shoot tissue concentrations of phosphorus. The slope of the TNC/P curve became positive in August showing increasingly higher values of TNC with higher shoot phosphorus. The slope of the starch/P curve in May was about twice of that of the soluble sugars/P ($b_{\text{starch}}/b_{\text{soluble sugars}} = 2$) indicating increasingly less reserve assimilates under improved major nutrient availability. Both soluble sugars and starch concentrations increased in a similar fashion in August with $b_{\text{starch}}/b_{\text{soluble sugars}} = 1.1$. There was no correlation between TNC concentration and tissue phosphorus in the roots either in May or August.

Discussion

There was a dramatic response to fertiliser addition by Cochlearia in +NPK and +NPKCa; and +Ca also caused detectable, but less pronounced changes. These responses were quantifiable in a number of ways. The responses in the population dynamics of Cochlearia displayed a varied pattern. Plants in the +NPK and +NPKCa showed an immediate increase in size and flowered in high numbers in 1992. In

contrast, +Ca prompted no immediate visible response, however there was higher increase, though statistically not significant, in the overall population size in 1992. Through the recruitment of large numbers of seedlings following the large seed output in 1992, overall population sizes exploded in +NPK and +NPKCa.

The depletion curves of the original cohorts showed a sharp change of course from a constant risk of death to an accelerated phase after the fertiliser addition in +NPK and +NPKCa. The largely semelparous character of Cochlearia resulted in the death of almost all reproducing individuals which is indicated by the one year time-lag after fertilisation. The non-flowering individuals did not suffer increased mortality. This indicates that there was no density- dependent regulation in operation, not to an extent which could have caused reduction in plant density through resource competition. It was anticipated that increased nitrogen supply would cause higher mortality over the winter period through prolonging the high uptake of nitrogen and shortening the period available for tissue maturing by building up metabolites for overwintering. As opposed to Henry et al.'s (1986) findings in arctic tundra where Cassiope tetragona and Dryas integrifolia plants were killed in the high (250 kg ha⁻¹) N, NPK plots in the winter, there was no significant increase in deaths in +NPK and +NPKCa. This is likely to be explained by the differences in the temperature climates and different rates of application in the two studies and also by different specific responses. Cochlearia at MK1.5 probably was able to respond promptly to the late addition of the fertilisers, complete growth and adjust the level of metabolites in its roots and shoots for overwintering. The extrapolation of the depletion curves for 1994 shows that the +NPK and +NPKCa cohorts are likely to have disappeared by 1995, owing to the reasons discussed above, while those of the control and +Ca are not likely to be depleted for

about another 5 years. There is more rapid turnover of individuals resulting from shorter life times in +NPK and +NPKCa. These, and the major shift in age distribution in the populations which received +NPK and +NPKCa, are in accord with the results of earlier work. Noble *et al.* (1979) found that there was a marked increase in overall population size and in shoot turnover in Carex arenaria on a fertilised sand dune. Also, there was an increased mortality and a major shift was apparent in the age distribution of the populations.

The above changes which affected both individual size (individual production) and density resulted in significant increases in plant cover. The original cover, which is about 5% on the skeletal soils of these ultramafics was increased to 23% in +Ca, and to about 39% in +NPK and 59% in +NPKCa. Cochlearia, the species with the highest density, was the main contributor to this increase (+Ca: 14%; +NPK: 20%; and +NPKCa: 28%). However, in two of the quadrats, the cover of grasses (Festuca rubra in one +NPK quadrat and Agrostis vinealis in one +NPKCa quadrat) also contributed to a large extent to the increase. There was an indication that if nutrient supply remained at the present level grasses may become dominant there and the initial large increases in the production (density, cover) of Cochlearia may be reversed in a similar fashion to that observed by Carter *et al.* (1987a) on the Keen of Hamar, Shetland, where graminoids outcompeted rarities after the initial success of the latter in a fertiliser experiment. In the +NPKCa at MK1.5 mosses established and reached an average cover of 11% by 1993.

The increase in production was not unexpected as a number of earlier fertilization experiments had resulted in large increases in plant biomass production on ultramafics. The addition of major nutrients brought about varied plant responses; sometimes N, P,

or K were effective alone, while in most cases a certain combination of them was required to achieve improved plant growth. Examples for field experiments can be found in Ferreira & Wormell (1971), Turitzin (1981), Carter *et al.* (1987a), Hobbs *et al.* (1988), and for glasshouse experiments in Proctor (1971), Proctor (1969), Jones *et al.* (1977), Cottam & Proctor (1982). All these seem to support the original view put forward by Gordon & Lipman (1926) that major nutrients were the main limiting factor on ultramafics.

However, there are other reports where the response was different in native and non-native species (Huenneke *et al.* 1990) and in several cases the addition of major nutrients did not improve plant growth on ultramafics (Blackshaw 1921, Vlamis 1949, Johnson *et al.* 1952, Walker 1954, Soane & Saunder 1959, Proctor 1971). Brooks (1987) explained these observations by saying that they were made on relatively nutrient-rich ultramafic soils. It should be noted, however, that in the field, fertilisation improves plant yields in most cases even on fertile soils (e.g. Bradshaw 1969).

The changes in vegetation development processes, population dynamics and production can all be related to physiological changes at the individual level. Phenological observations suggested that +NPK and +NPKCa were likely to have alleviated stress as plants receiving these treatments rapidly turned green from reddish-brown. This in turn might have induced earlier maturing of Cochlearia whereby plants reached the reproductive stage and completed their life cycles more rapidly than in the control and +Ca treatments. Rosette diameters were significantly higher in +NPK and +NPKCa than in either control, or +Ca. The number of leaves per rosette was also different, though a significant difference was found between +NPK and +NPKCa, with the former being more leafy. It may be that this difference is responsible for the significantly lower

production of flower buds by 12-month old plants. This seems to support those who view phenological (developmental) stage structure of equal importance to age structure for population dynamic processes (Law 1983). This is not to deny the usefulness of age structure which under some circumstances may be directly related to phenological stage. Certainly plants' ability to respond plastically to changed resource availability is of paramount importance in the early stages of colonisation and differences in species' ability can determine to a large extent the outcome of vegetation shaping processes (Grime 1979, 1988, Campbell & Grime 1991).

The much increased seed production, partly through the mass flowering of the adults and partly due to the early maturing of the 1992 recruitment in the second year after fertilisation, allowed an explosion in the populations without increased seedling mortality. It is suggested therefore that the carrying capacity of the +NPK and +NPKCa quadrats has not yet reached saturation. In other words, density-dependent regulation has not reached its maximum and if operational it has more influence on individual yield than fate. Further aspects of the carrying capacity of the ultramafic outcrops are discussed in Chapter VI.

The dramatic increase in the proportion of flowering individuals and the resultant high seed production, the increase in size and change in colour from reddish-brown to green were the overall responses to the combination of fertilisers. The small-scale addition experiment set up in June 1993 confirmed that phosphorus was the major limiting nutrient. Plants in +P showed all the changes which were apparent in +NPK and +NPKCa earlier and there was no response to +N and +K alone. For optimum growth Marschner (1986) quotes the range of 0.3-0.5% of tissue dry weight as the phosphorus requirement which varies with species and soil supply. The concentration of phosphorus

in the shoots of Cochlearia in +NPK and +NPKCa was in that range, while there was less in the control and +Ca plants. Marschner (1986) lists manifestations of inadequate phosphorus supply which include retarded growth often with reddish coloration; reduced leaf cell division and expansion; and lowered rates of photosynthesis and respiration. As inorganic phosphorus has a vital role in regulating photosynthesis and carbohydrate metabolism and translocation in the leaves, its availability has a decisive role on plant growth. It has been shown, for example, for Glycine max that an adequate supply of phosphorus was of major importance in determining the starch:sucrose ratio in the leaves and the allocation of photosynthates to developing fruit (Giaquinta & Quebedeaux 1980). A close relationship was found between phosphorus supply and the number of flowers in apple (Bould & Parfitt 1973) and the time of flower initiation in Trifolium subterraneum (Rossiter 1978).

Henry *et al.* (1986) found that increases in the flowering of dicotyledonous species in a tundra fertiliser addition experiment occurred after NPK application only, N alone did not increase flowering. Several other workers reported dramatic responses to N or P application or both in arctic graminoids and forbs (McKendrick *et al.* 1980, Babb 1973, Babb & Whitfield 1977).

The findings of this study suggest that the physiological changes prompted by increased uptake of phosphorus were the major causal factor for the changes at the individual and population levels. It is suggested that the availability of phosphorus for uptake by plants may be limited by slow mineralisation of phosphorus caused by low temperature. The results of the carrying capacity study showed that plants sown at 100 seeds per 0.01 m² were vigorous and green when grown in soil previously stored for 2 y in a growth room at 15-20 °C. Seeds sown at the same density at MK1 were all similar to naturally

emerged seedlings - made very little growth and were reddish-brown in colour. It is less likely that the low temperatures of this high altitude environment would themselves be controlling root growth thereby limiting the capacity of the Cochlearia to balance nutrient uptake. This is further supported by the increased uptake of all mineral nutrients investigated in +Ca. Therefore the unavailability of phosphorus due to soil chemistry is a more plausible explanation. Some workers stressed the possible interaction between phosphorus and nickel, resulting in decreased availability of phosphorus on some ultramafic soils (Soane & Saunder 1959, Vergnano 1959, Jeffrey 1971). Other elements (e.g. iron) were also suggested as reducing phosphorus uptake (Russel 1954). Bulusu et al. (1978) showed that soluble phosphates had a high affinity for serpentine and that could also contribute to low phosphorus availability. However, the higher uptake of phosphorus on the addition of calcium itself may suggest that root growth became less restricted and plants were able to take up more phosphorus. Carter et al. (1987b) found that although total soil phosphorus was not low, plants responded to phosphorus addition in the field. They considered that the mobile pool was restricted due to the possible formation of nickel and iron phosphates, which rendered phosphorus unavailable to plants. They also supposed that the shallow skeletal soil on their Shetland ultramafic site was susceptible to drought and this, with low phosphorus availability may have seriously stressed seedlings through the inhibition of root growth, thus impairing their establishment and inhibiting the development of full vegetation cover in a similar way to that envisaged for Arrhenatherum elatius on a Derbyshire limestone by Grime & Curtis (1976).

The significantly higher nitrogen concentrations in +NPK and +NPKCa were certainly also of importance for increased production, as the nitrogen nutrition of plants is of

central importance for plant photosynthetic production (Chapin *et al.* 1987, Tieszen 1978). Although direct measurements on photosynthetic capacity were not carried out, there is an indication from the TNC allocation pattern in the different treatments that photosynthetic carbon assimilation was limited in non-fertilised plants. The decrease in TNC in May in the fertilised plants as opposed to the controls is indicative of sink limitation in the latter. It has been long established that plants growing in alpine environments achieve rapid growth in spring by mobilising their carbohydrate reserves, especially those of the roots and rhizomes (Fonda & Bliss 1966, Mooney & Billings 1960) therefore a further strong indication of sink limitation in the controls is the much lower decrease in total root TNC in comparison with either of the treated ones. The lower total shoot TNC content in the fertilised plants on the other hand can be interpreted as an increased use of assimilates for growth rather than storage; the comparison of rosette sizes in twelve-month old plants showed that growth did occur. The high negative correlation in May between shoot phosphorus concentrations and TNC and also starch is a further supporting evidence. This is consistent with findings in earlier fertiliser experiments where fertilisation of arctic tundra vegetation resulted in decreased concentrations of plant TNC suggesting that sink limitation was alleviated and carbohydrates were used for growth rather than for storage (Chapin & Shaver 1985, Shaver & Chapin 1980, McKendrick 1978).

The high increase in TNC and similarly in starch both in the roots and the shoots in August suggested that the plants were completing their growth and were accumulating carbohydrate reserves for overwintering. The higher shoot TNC in the +Ca, and especially +NPK and +NPKCa plants in August compared with the control indicated that ameliorated mineral nutrition has increased the capacity of the plants to accumulate

more carbohydrate; the ratio of shoot starch:root starch did not differ however between treatments. The high values (> 1) for the ratio of shoot starch:root starch at both samplings conflict with reports on perennial species where this ratio was generally less than one (e.g. Fonda & Bliss 1966). As Cochlearia is a winter-green species and in optimum conditions is biennial it is just possible that these may satisfactorily explain the observed high shoot starch concentrations. Further work is underway to follow the annual cycle of carbohydrate allocation in Cochlearia in the different treatments.

Increased potassium concentrations in fertilised plants are thought to have also contributed to growth in a complex manner by assisting the adjustment of the osmotic balance and also contributing to winter hardiness (Marschner 1986, Alden & Hermann 1971).

The role of calcium in improving the mineral nutrition of Cochlearia on this ultramafic soil, which had been found highly toxic to non-ultramafic races, is not clear from this study. Although +Ca increased the tissue concentrations of nitrogen and phosphorus there was no response in rosette size, number of leaves and flower development. It is possible that though the increases in tissue concentration of the two elements were significant, no dramatic responses followed, for these concentrations were still below a certain 'threshold' value. The cover data, however indicate that the vegetation underwent some development over the two years following calcium addition. This may be due to an effect on seedling establishment through improving resource capture at a critical stage. However it is also possible that 1991 fortuitously coincided with higher seed production in the +Ca quadrats and the perceived increase in cover would have happened without calcium fertilisation.

A common feature of most ultramafic soils is their high magnesium concentration and

in many cases they are also high in nickel. The soil at the Meikle Kilrannoch outcrops has been considered as highly toxic and inhibitory to the growth of non-adapted species (Proctor & Woodell 1971). Magnesium and to a lesser extent nickel were suggested as likely causes of toxicity (Proctor 1971, Johnston & Proctor 1981). Soil and plant tissue analyses in the present study indicated no fertiliser effect on magnesium uptake and Mg/Ca quotients changed only as a result of higher calcium uptake in +Ca and +NPKCa. Nickel concentrations just fell short of being significantly different between the control and +Ca and the +NPK and +NPKCa treatments, the latter pair containing about half the amount taken up by control and +Ca plants. +Ca alone seem to have increased nickel uptake by about 30%. This increased nickel uptake in the shoots may have cancelled the effects of extra tissue nitrogen and phosphorus in the +Ca plants. Although nickel toxicity has been demonstrated in Cochlearia grown in a hydroponic experiment (Chapter IV) it is not known how nickel may interfere with physiological processes when present at concentrations which are not acutely toxic.

It appears that although the range of species which can tolerate ultramafic soils is limited, those which do occur are also limited in their ability to develop closed vegetation on the skeletal soils at MK1.5 (and possibly on the other outcrops also) mainly because of the relatively small numbers of seeds. This may arise from a quasi equilibrium: the carrying capacity of the site, despite the low above-ground production, may be fully exploited at the prevailing resource conditions by the plants present in a similar manner to that which Spence (1957) suggested in his study on Unst, Shetland. The results of the effects of +NPK and +NPKCa point into this direction. No evidence was obtained that toxicity by magnesium, or nickel inhibited vegetation development, though it should be borne in mind that the sizes of the experimental quadrats do not

allow inferences on spatial variation and its effects on spatial dynamics of vegetation development. Also it should be mentioned that the results on population dynamics and production are somewhat artificial as herbivores were excluded.

Chapter VIII

Concluding remarks

This series of studies intended to investigate: (a) soil - plant correlations on the skeletal soil of the MK1 ultramafic site to offer explanations for the spatial distribution of the species (which include two important rarities); (b) to use the field observations to formulate hypotheses to be tested in solution cultures about causality; (c) to investigate the effects of various solution iron and nickel concentrations on photosynthesis in different Cochlearia species; (d) to use a perturbation experiment to study the limiting factors to the development of closed vegetation over skeletal soil; (e) to rephrase vegetation development in terms of site carrying capacity at MK1; and (f) to study the population dynamics of Lychnis alpina to provide base-line data for the long-term monitoring and conservation of this national rarity.

The study of the small-scale spatial variation of soil properties and vegetation patterning suggested that different physical and soil chemical factors controlled the distribution of different species. Slope appeared to be of a factor of major importance, probably through providing different availabilities of ions for uptake by plants. The use of ion-exchange resin bags had been chosen to reflect ion availability as a function of the amounts of ions present and soil water content. It could be shown that the concentrations of ions was higher in the wetter areas down-slope. The high negative correlation of Cerastium fontanum ssp. scoticum with soil magnesium in the field was substantiated in the growth room study, where its growth was less in the presence of high concentration of magnesium in culture solutions. The indifference to soil chemistry

of Festuca rubra was also supported by the results of the culture experiments. The results for Cochlearia pyrenaica ssp. alpina were more difficult to interpret. The field study suggested a high correlation between the occurrence of this species and high soil nickel and higher cation availability in general. In contrast, the solution culture experiments showed that the addition of nickel caused reduced growth. The hypothesis cannot, however, be said to be refuted because there was only one nickel concentration used (apart from the control) and no simulation of a nickel gradient. Also, the culture solutions were based on Johnston & Proctor's (1981) work and not on the analytical data collected in the present study (and used in the ordination). Future work should compare the efficacy in simulating real-soil conditions of the solutions suggested by Johnston & Proctor (1981) and of a different one based on the present analyses (Chapter II). The findings of the study on the photosynthetic responses of different Cochlearia species also proved to be difficult to interpret, mainly because the running of the experiment, especially the measurements relating to the characteristics of photosynthetic system in the species was fraught with technical difficulties. A carefully planned repetition of the experiment with higher numbers of replicates using a more finely tuned range of solution nickel concentrations is likely to successfully identify sites of interference by nickel in the photosynthesis.

There were clear and dramatic short-term responses by Cochlearia and other species to perturbation (nutrient addition). It would be of interest, especially for site conservation, to continue the study. The comparative ecophysiology of the species in nutrient-sufficient and nutrient-limited conditions would add to our understanding of ultramafic tolerance (a term which implies tolerance to soil chemical stress compounded by other climatic and site specific stress factors). Extending the study to allow herbivores to

interact would require a far larger-scale study, which does not seem feasible because of the limited extent of the ultramafic areas at Meikle Kilrannoch. For conservation it would be of prime interest to include Lychnis alpina in a nutrient addition experiment to enable the study the species' responses to changed community dynamics.

The work on the carrying capacity of the MK1 site (Chapter VI) needs further elaboration of methodology and probably has a wider interest beyond the confines of ultramafics.

The work on the population dynamics of Lychnis alpina will continue within the scope of the Monitoring of Rare Plants in Scotland project, which is co-ordinated by the Royal Botanic Garden, Edinburgh and Scottish Natural Heritage.

The information accumulated over the years about the ecology of the site should provide a sound basis for further research as outlined above. Also, the investigation of other areas such as the possibility of genetic adaptation to local small-scale variation in the environment at the site and the biochemical basis for tolerances to the compound ultramafic stress factor are needed.

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Appendix I

Intra-site variation in the demography of Lychnis alpina L. on MK1

Summary

- (1) The demography of Lychnis alpina L. on skeletal soil at the MK1 ultramafic site is reported.
- (2) Estimates were made of seed production and the size of the soil seed bank at different times. The germination of seeds was investigated in a laboratory experiment, seedling survival, numbers and sizes of rosettes per genet, flowering, and herbivory at flowering were recorded in permanent plots in the field.
- (3) There were differences in the annual rate of increase of the population in two areas of the outcrop; $\lambda=1.58$ on deeper soil, indicating a slightly increasing population and $\lambda=1.05$ indicating a stable population on shallow soil over outcropping bedrock. The high survival of the original cohort after two years (0.8) suggests that it is the longevity of established plants rather than enhanced recruitment that is responsible for the increase of the deeper-soil population.
- (4) High rates of herbivory occurred at flowering and the soil seed bank was found to be small.
- (5) The longevity of L. alpina warrants further study to obtain data in the medium-term to long-term dynamics of the population at Meikle Kilrannoch.

Introduction

There has been an increasing awareness of the limitation that minimum population size can have on the viability of a population (Lamont & Klinkhamer 1993). It is recognised as imperative that work on the conservation of plant communities with rare species focuses on the biology of these species and their autecology in relation to local community processes to obtain a realistic assessment of species requirements. The study of the life history of a species in relation to environmental variation may reveal specific conditions which indicate increased vulnerability or population stability. There has been a number of reports which showed temporally or spatially dissimilar patterns of recruitment, mortality, survival, and reproductive allocation between populations of the same species (Matlack 1987, Mack & Pyke 1983).

This chapter reports the intra-site variation in the demography of Lychnis alpina at the Meikle Kilrannoch ultramafic site. Estimates were made of seed production and the size of the soil seed bank at different times. Recruitment, death and survival of adult and seedling cohorts with numbers and sizes of rosettes per genet, flowering and herbivory at flowering were recorded in permanent quadrats in the field.

Lychnis alpina is a very rare native species of the British flora restricted to two sites. One of them is at Hobcarton Crag in Cumberland, England and the other is the Meikle Kilrannoch site. The size of the population at Hobcarton Crag was reported about only 150 plants (Proctor & Johnston 1977), while there is a much larger population of about 65,000 specimens at Meikle Kilrannoch (Proctor et al. 1991, L. Nagy unpublished).

Lychnis alpina is a herbaceous perennial plant with profusely branching tap-root. A woody stock, branching, or single, produces 12-18 rosette leaves of 12-18 mm in length

and 4-5 mm wide. The rosette leaves are upright (or at an acute angle) when newly sprung and flatten out over the previous year's dead or dying leaves by the end of the growing season. Flowering is usually in late June - early August; stems bear inflorescences of 1-7 flowers. The main pollinators on Meikle Kilrannoch are Bombus species and certain diptera e.g. Bibionidae (personal observation). Self pollination also occurs (Clapham, Tutin & Moore 1987). Flowering and seeding plants either produce new rosettes, or alternatively they die after seed maturation. The total number of viable seeds per capsule varies widely. Capsules open with five teeth. The small (< 2 cm height) stature of the rosettes affords protection from grazing animals, however flowering stems often suffer herbivory.

Lychnis alpina has an amphi-atlantic distribution with a subarctic-alpine occurrence, often on ultramafics, or metalliferous sites.

Materials and Methods

STUDY SITE

The study was carried out at the ultramafic outcrop at MK1 site described in Chapter I.

RECORDING OF PLANTS

Nine quadrats of 50 cm x 42 cm were set up on 5 May 1991 at the north end of MK1, where Lychnis occurs abundantly. Five of the quadrats were near the northernmost tip

of the site (area 1) and the other four due south of the first group in an area with shallow soil over outcropping ochreous bedrock (area 2). The two areas are separated by a stretch of blanket bog of about 30 m in width (Fig. 6.1).

The same method was used for recording the position and following the fate of each Lychnis plant as was described in Chapter VII for Cochlearia pyrenaica ssp. alpina. The rosettes could not be aged because of the wide variation that the plants exhibited in response to micro-environment.

Data were collected on herbivory as plants suffered a heavy loss of flower heads at flowering.

ROSETTE SIZE AND FLOWERING

Rosette sizes and flowering (with the number of flowers in individual inflorescences) were recorded in a separate area, 3 m x 3 m in size, at the southern end of the MK1 outcrop (Chapter II) in 1992 to investigate the relationship between rosette size and reproductive ability.

FRUIT AND SEED PRODUCTION

Eighteen capsules were collected in September 1991 and the number of seeds per capsule was counted. Filled and sterile seeds were separated. Seed production was calculated as a product of the number of capsules times average number of filled seeds per capsule.

SEED BANK

Soil cores of 2 cm x 2 cm were collected at 4-5 cm depth from around five mature individual *Lychnis* plants, which had been marked in the area preceding the sample collection. Sampling was carried out on 14 October 1993. The February samples were thought to be more representative of the growing season conditions and numbers; the July samples were thought to be more representative of the seedling flush in May-June. The July samples were thought to be more representative of available seeds. Four cores were taken from each of the five plants. The 5 cm samples were divided into 1 cm sections and the seeds were dispersed, and the seeds were counted. The soil was then sieved through a 2 mm sieve to remove any remaining seeds. The seeds were then counted for seeds of *Lychnis*. No seeds of *Lychnis* were found in the soil.

Results

POPULATION DENSITY

The density of *Lychnis* plants in the area is shown in Table 1. There was a large increase from 169 to 267 individuals in the area at northern tip while there was practically no change in the density (127 and 139) of plants in the area over shallow soil over the two years. These figures are reflected in the annual rates of population increase (15.9%) for the population in the area in 1993 (Table 2). The values

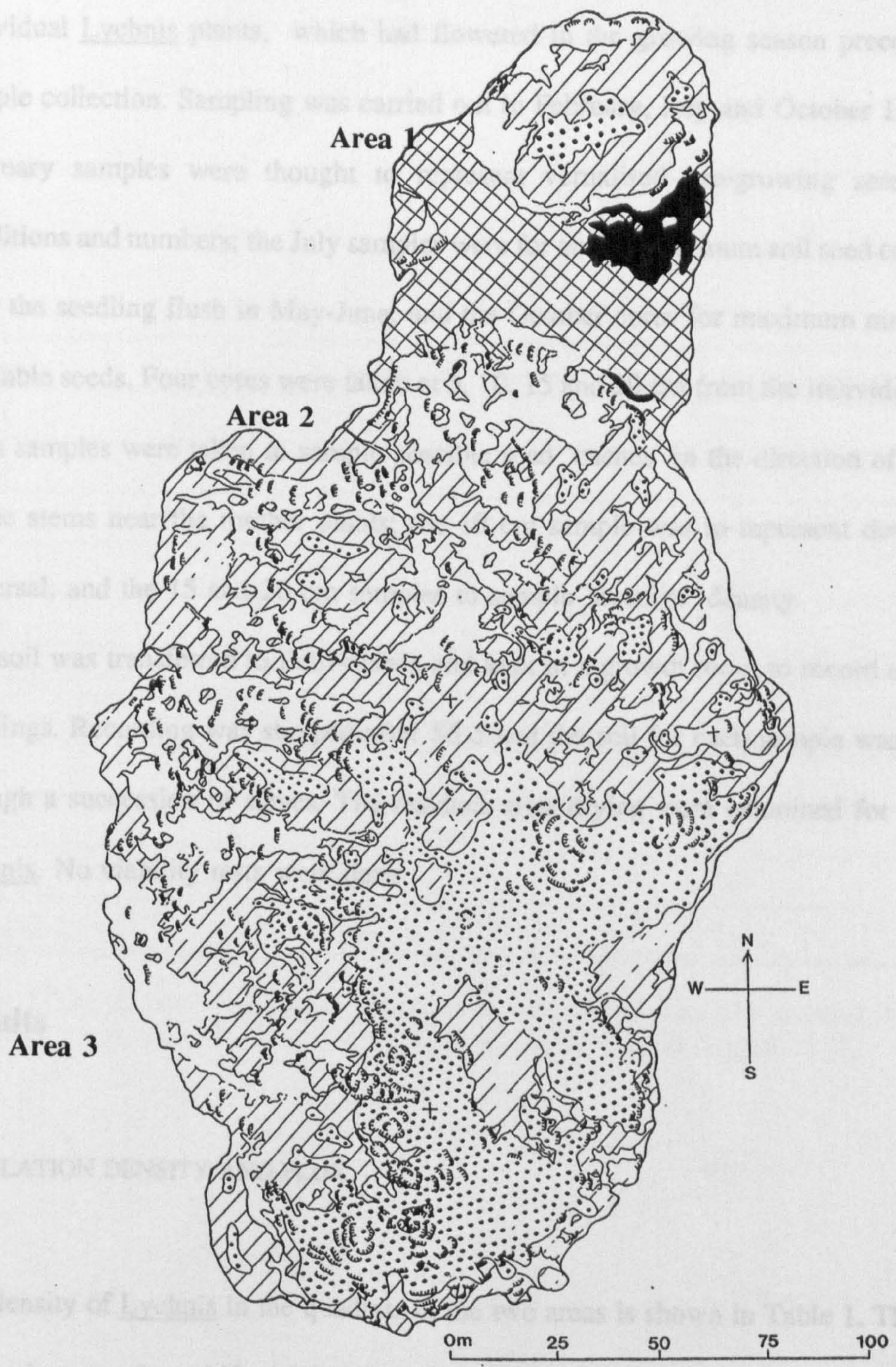


Fig 6.1 Map of MK1 showing the location of areas 1-3 where the estimates of potential carrying capacity (PCC) and realisable carrying capacity (RCC) were made in 1993.

Soil cores of 2 cm x 2 cm were collected at 4-5 cm depth from around five mature individual Lychnis plants, which had flowered in the growing season preceding the sample collection. Sampling was carried out in February, July and October 1993. The February samples were thought to represent vernalised pre-growing season seed conditions and numbers; the July samples were for annual minimum soil seed conditions after the seedling flush in May-June; and the October cores for maximum numbers of available seeds. Four cores were taken at 5, 10, 15 and 20 cm from the individuals. The 5 cm samples were taken to sample possible seed 'caches' in the direction of the lean of the stems near the mother plants; the 10 cm sample was to represent down-slope dispersal; and the 15 and 20 cm samples to sample 'average' density.

The soil was transferred to Petri-dishes and kept in a growth room to record emerging seedlings. Recording was stopped after 56 d and the soil for each sample was washed through a succession of sieves. The residues after drying were examined for seeds of Lychnis. No viability tests were done.

Results

POPULATION DENSITY AND FLUX

The density of Lychnis in the quadrats in the two areas is shown in Table 1. There was a large increase from 169 to 267 individuals in the area at northern tip while there was practically no change in the density (129 and 134) of plants in the area over shallow soil over the two years. These figures are reflected in the annual rates of population increase ($\lambda=N_t/N_{t-1}$) for the two areas: $\lambda_{\text{area 1}}=1.58$; $\lambda_{\text{area 2}}=1.05$ (Table 2). The values

	August 1991 (plants/m ²)	August 1993 (plants/m ²)
Q11	276	457
Q13	195	243
Q14	242	242
Q15	114	133
Q16	19	261
Average for area 1	169	267
Q17	119	142
Q18	176	176
Q19	133	143
Q20	86	76
Average for area 2	129	134

Table 1 The density of Lychnis alpina in individual quadrats and average values (in bold) for the two areas are also shown for August 1991 and August 1993.

Period	Q11	Q13	Q15	Q14	Q16	Q17	Q18	Q19	Q20
Jun-Aug '91	1.0	1.21	1.71	1.13	1.00	1.56	1.12	1.27	1.13
Aug-Jun '92	1.16	1.54	1.08	1.10	9.5	0.88	0.86	1.07	0.94
Jun-Aug '92	1.12	1.03	1.46	1.09	0.71	1.05	1.25	1.10	1.06
Aug-Jun '93	1.13	0.85	0.71	0.89	1.67	1.13	1.03	1.00	1.00
Jun-Aug '93	1.13	0.93	1.04	0.94	1.22	1.15	0.90	1.00	0.89
Jun '91-Aug '93	1.65	1.5	2.00	1.13	13.75	1.86	1.12	1.36	1.00
Aug '91-Aug '93	1.65	1.24	1.16	1.00	13.75	1.20	1.00	1.07	0.89

Table 2 Finite rate of population increase ($\lambda=N_t/N_{t-1}$) for periods of the growing seasons in 1991-1993 and over the whole period.

for λ for individual quadrats varied between 0.89 and 1.65 (13.75). Extreme values were found for Q16 where the final value of density was fourteen times of initial one. This was caused by a large number of seedling recruits following the production of a high number of seeds in 1991.

Population flux was also different in the two areas (Fig 2). Mortality was higher in proportion to recruitment in area 2 and only a slight net increase occurred. In area 1, an increase in net plant numbers about 4-5 times higher than in area 2 was a result of both higher recruitment and lower death.

There were large differences in the percentage of individuals flowering in the two areas in 1992 and 1993. In area 1, 10.4% and 7.9% of the plants flowered as opposed to 16.8% and 11.3% in area 2. No difference was found in 1991 when 11.0% flowered in area 1 and 11.5% in area 2 (Table 3).

Herbivory damage to the flowering plants by clipping off the flower heads, probably by small vertebrates, was about 30% in both areas in 1991 (Table 3). In contrast, a much higher percentage of the flowers became destroyed by unknown herbivores in area 2 in 1992 and 1993 (48% and 54% versus 34.6% and 33.3%).

Longevity of cohorts

Figure 3 shows the survival of plants in the Lychnis alpina cohorts first recorded in June 1991. There was little change in the size of either of the cohort over the three growing seasons - 80% of the plants survived from the first recording (Tables 4 and 5). The highest mortality occurred between the end of one growing season and the beginning of the following: 7% and 5.8% in area 1 and 5.9% and 4.6% in area 2

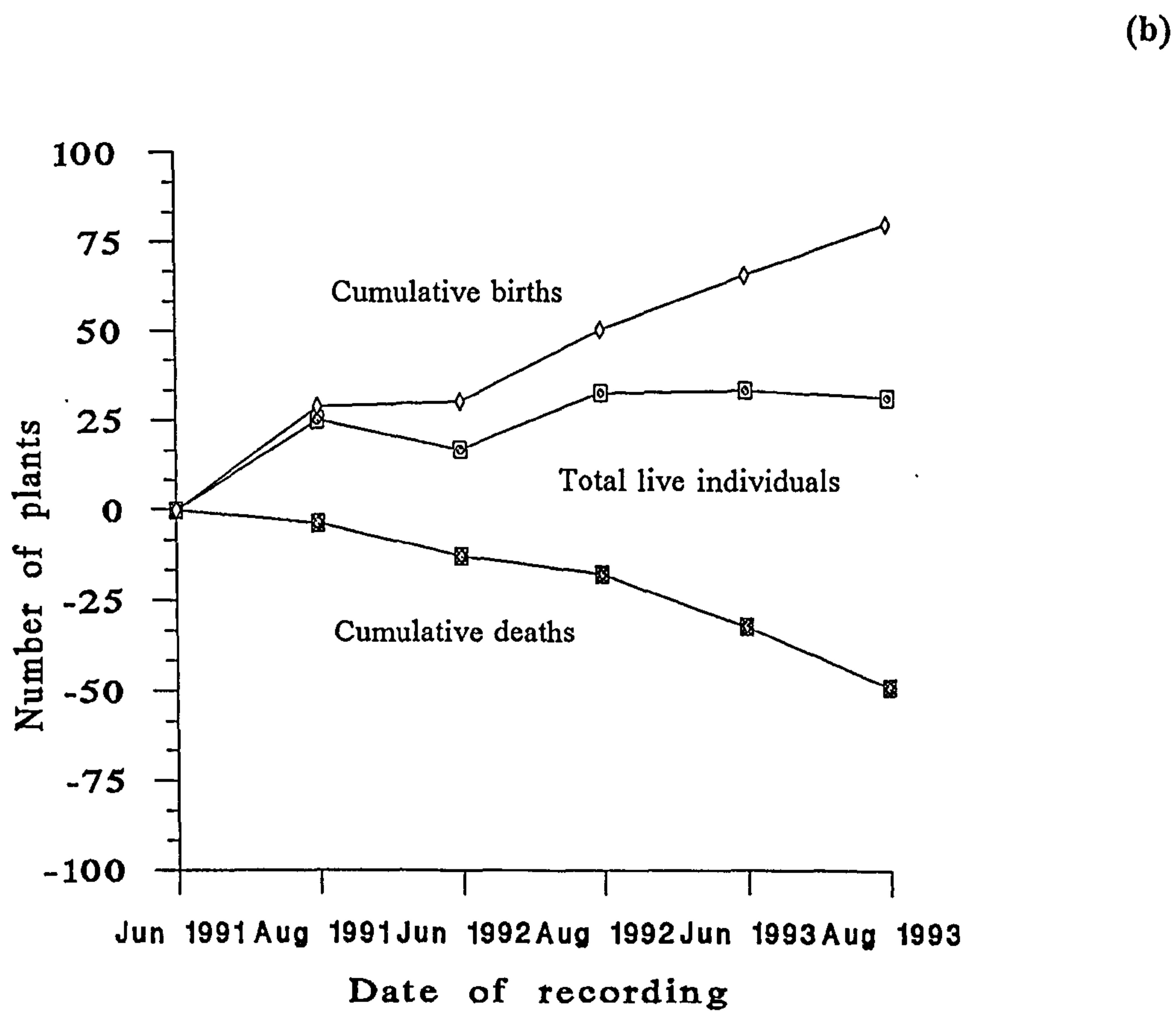
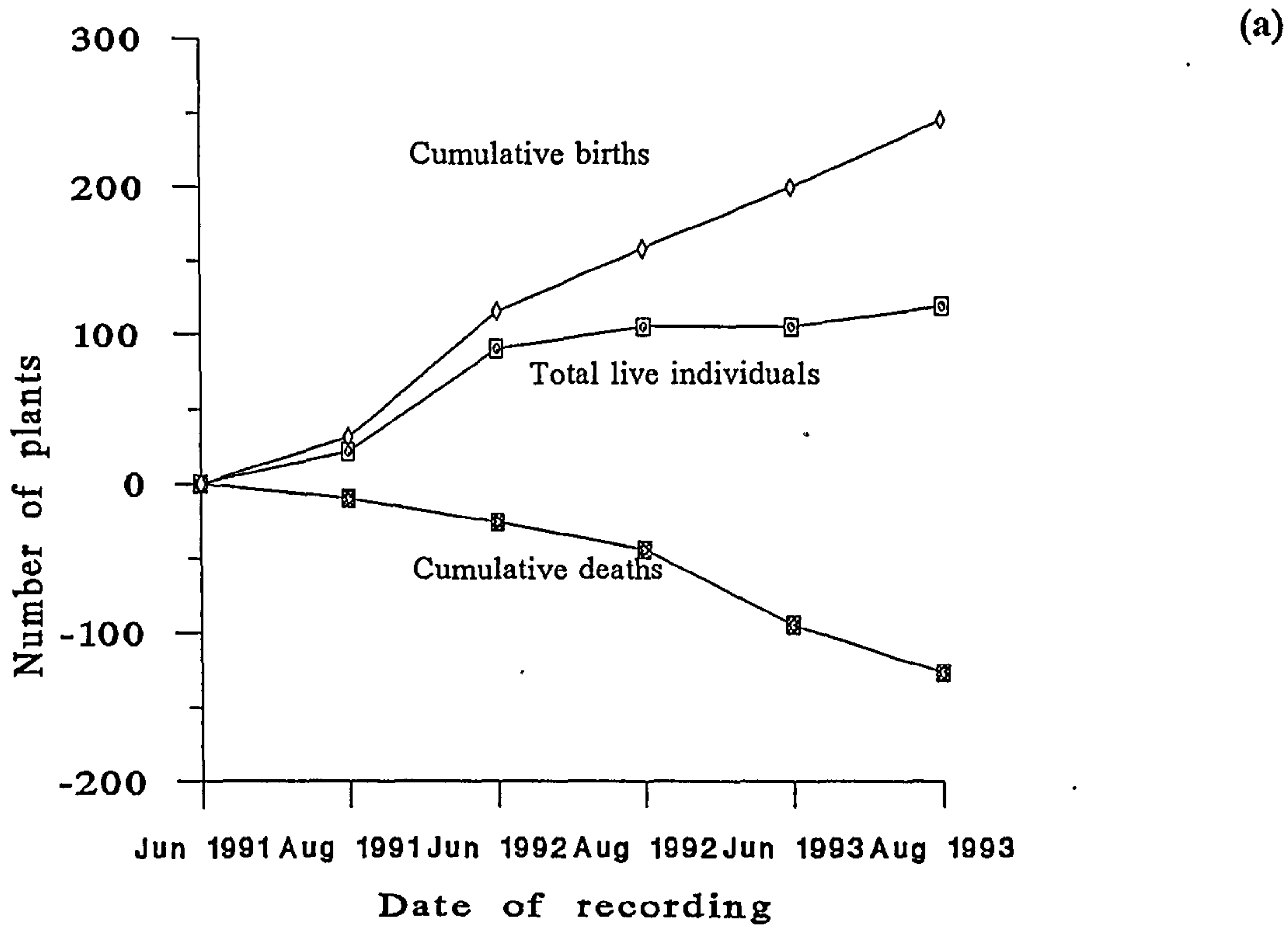


Fig 2 Population flux in *Lychnis alpina* in areas 1 (a) and 2 (b) at Meikle Kilrannoch between June 1991 and August 1993.

Year	Flowering (% of total)		Herbivory (% of flower stalks)	
	Area 1	Area 2	Area 1	Area 2
1991	11.0	11.5	35.3	30.0
1992	10.4	16.8	34.6	48.0
1993	7.9	11.3	33.3	54.0

Table 3 Percentage of flowering Lychnis plants in areas 1 and 2 at MK1 in the years 1991-1993. The right hand columns show the percentage of flower stalks destroyed by herbivores.

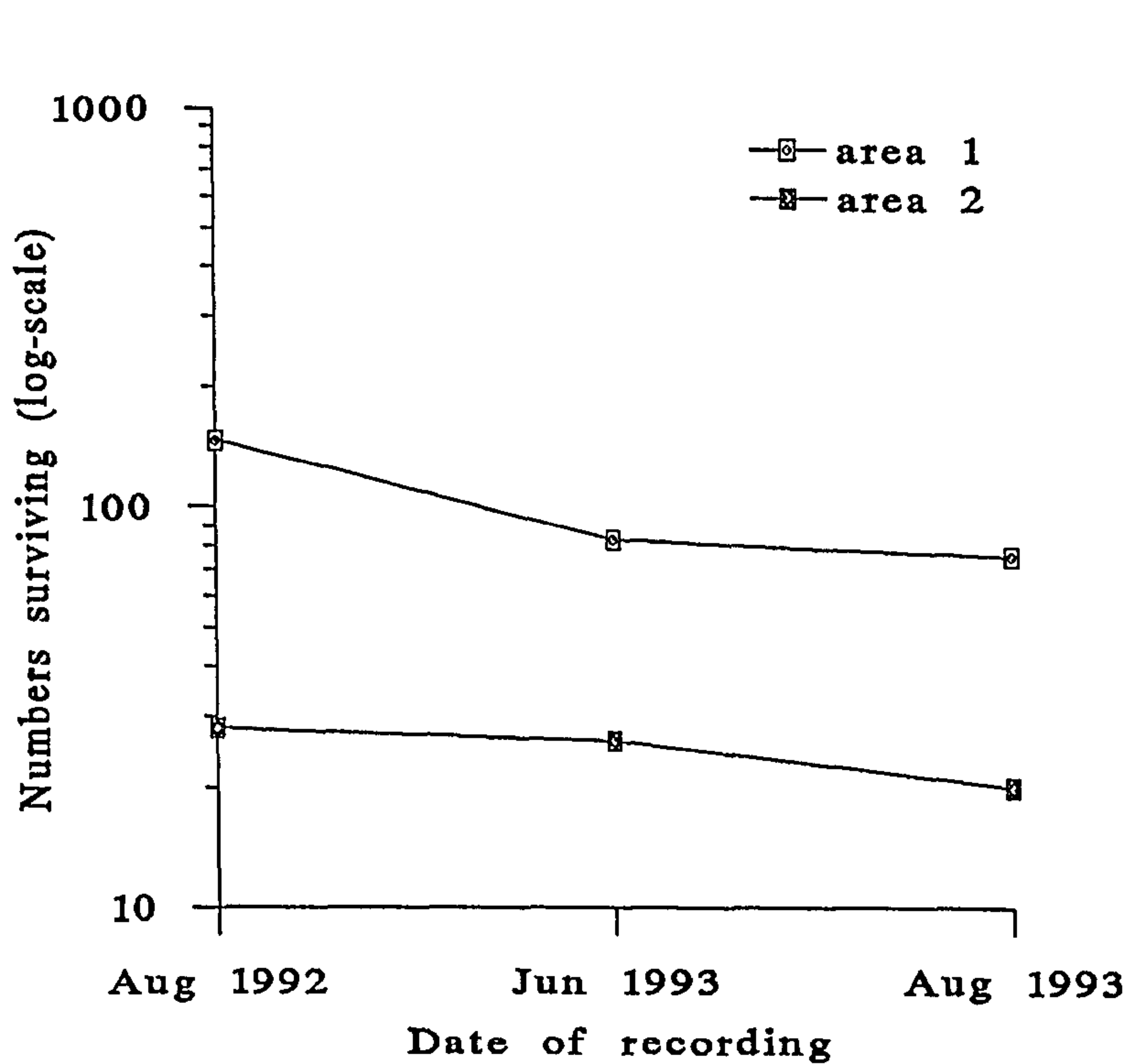
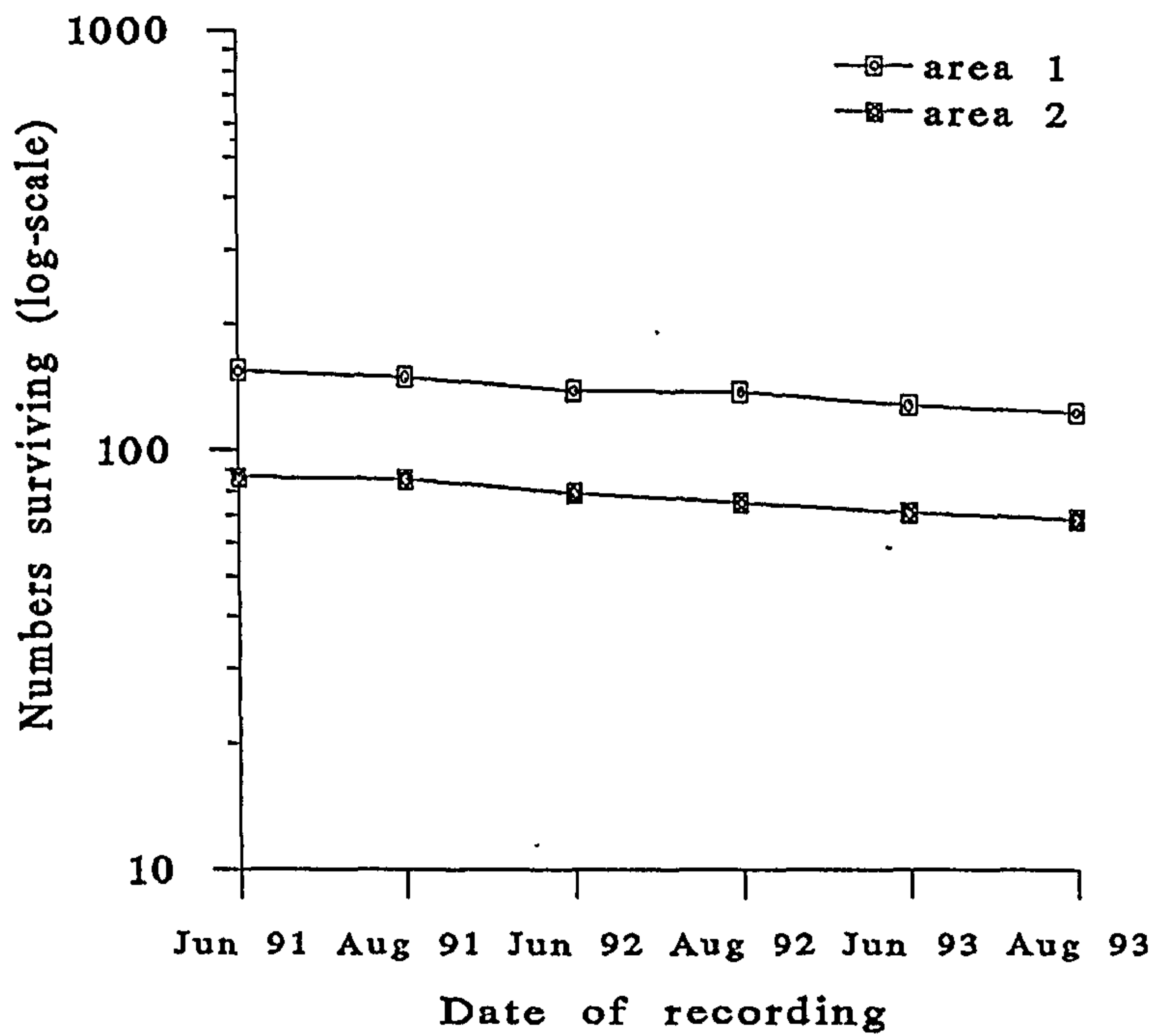


Fig 3 Survivorship curves for the cohorts of *Lychnis alpina* in areas 1 and 2 at Meikle Kilrannoch first recorded on 26 May 1991 (a) and for seedlings in areas 1 and 2 recruited in 1992 (b).

Age (period of time) x	Number N_x	Survival l_x	Mortality d_x	Mortality rate $q_x = (d_x/l_x)$	Survival rate $p_x = 1 - q_x$	Seeds/plant m_x	Reproductive value V_x^*
Jun 1991	155	1.000	0.033	0.033	0.967	-	-
Aug 1991	150	0.967	0.070	0.072	0.928	7.2	24.7
Jun 1992	139	0.897	0.007	0.008	0.992	-	-
Aug 1992	138	0.890	0.058	0.065	0.935	10.2	18.9
Jun 1993	129	0.832	0.032	0.038	0.962	-	-
Aug 1993	124	0.800	-	-	-	9.7	9.7+..

Table 4 Life table and fecundity schedule for cohorts of the Lychnis alpina population in area 1 at MK1. Recording began on 21 May 1991 and the last recording was on 4 September 1993. (* $V_x = m_x + \sum_{i=1, \infty} (l_{x+i}/l_x)m_{x+i}$)

Age (period of time) x	Number N_x	Survival I_x	Mortality d_x	Mortality rate $q_x = (d_x/I_x)$	Survival rate $p_x = 1 - q_x$	Seeds/plant m_x	Reproductive value V_x^*
Jun 1991	86	1.000	0.012	0.012	0.988	-	-
Aug 1991	85	0.988	0.059	0.060	0.940	14.1	34.5
Jun 1992	79	0.919	0.047	0.051	0.949	-	-
Aug 1992	75	0.872	0.046	0.052	0.948	15.1	22.5
Jun 1993	71	0.826	0.024	0.029	0.971	-	-
Aug 1993	69	0.802	-	-	-	8.0	7.4+..

Table 5 Life table and fecundity schedule for cohorts of the Lychnis alpina population in area 2 at MK1. Recording began on 21 May 1991 and the last recording was on 4 September 1993. (* $V_x = m_x + \sum_{i=1, \infty} (I_{x+i}/I_x)m_{x+i}$)

compared with 0.7% and 3.3% in area 1 and 1.2% and 4.7% for the summer periods. The reproductive value of plants in area 1 was lower in both 1991 and 1992 than in area 2 (34.5 and 22.5 versus 24.7 and 18.7). In 1993, the value for area 1 was slightly higher than for area 2 (9.7 versus 7.4).

Seedling survival for the 1992 cohort was different in the two areas (Fig 3b). There was a much higher mortality over the winter in area 1 (43.8%) than in area 2 (7.1%). Conversely, a higher rate of mortality occurred in the summer in area 2 (21.5%) with shallower soil than in area 1 (4.8%).

ROSETTE SIZE VERSUS FLOWERING

The average diameter of the rosettes of the flowering specimens was significantly higher (24.7 ± 3.23 mm, $p = 0.05$) than in the non-flowering ones (13.8 ± 3.23 mm). It is noticeable, however, that only 61% of the individuals within the higher size range flowered. No correlation was found between rosette size and the number of flowers in inflorescences (Fig. 4).

FRUIT AND SEED PRODUCTION

The total number of seeds per capsule varied between 0-83 with 28% of the capsules containing less than 10 seeds. The average number of filled seeds was 31 per capsule with 38% of the capsules with less than 10 seeds. There was a large variation in fruit and seed production in the two areas and over the three years with no consistent pattern (Table 6).

Year	Average number of capsules/quadrat		Estimated number of seeds/quadrat	
	Area 1	Area 2	Area 1	Area 2
1991	4.6	9.8	141	299
1992	9.8	9.3	301	284
1993	6.0	4.5	184	138

Table 6 Average number of capsules and seeds produced by Lychnis in quadrats in the two areas in the years 1991-1993.

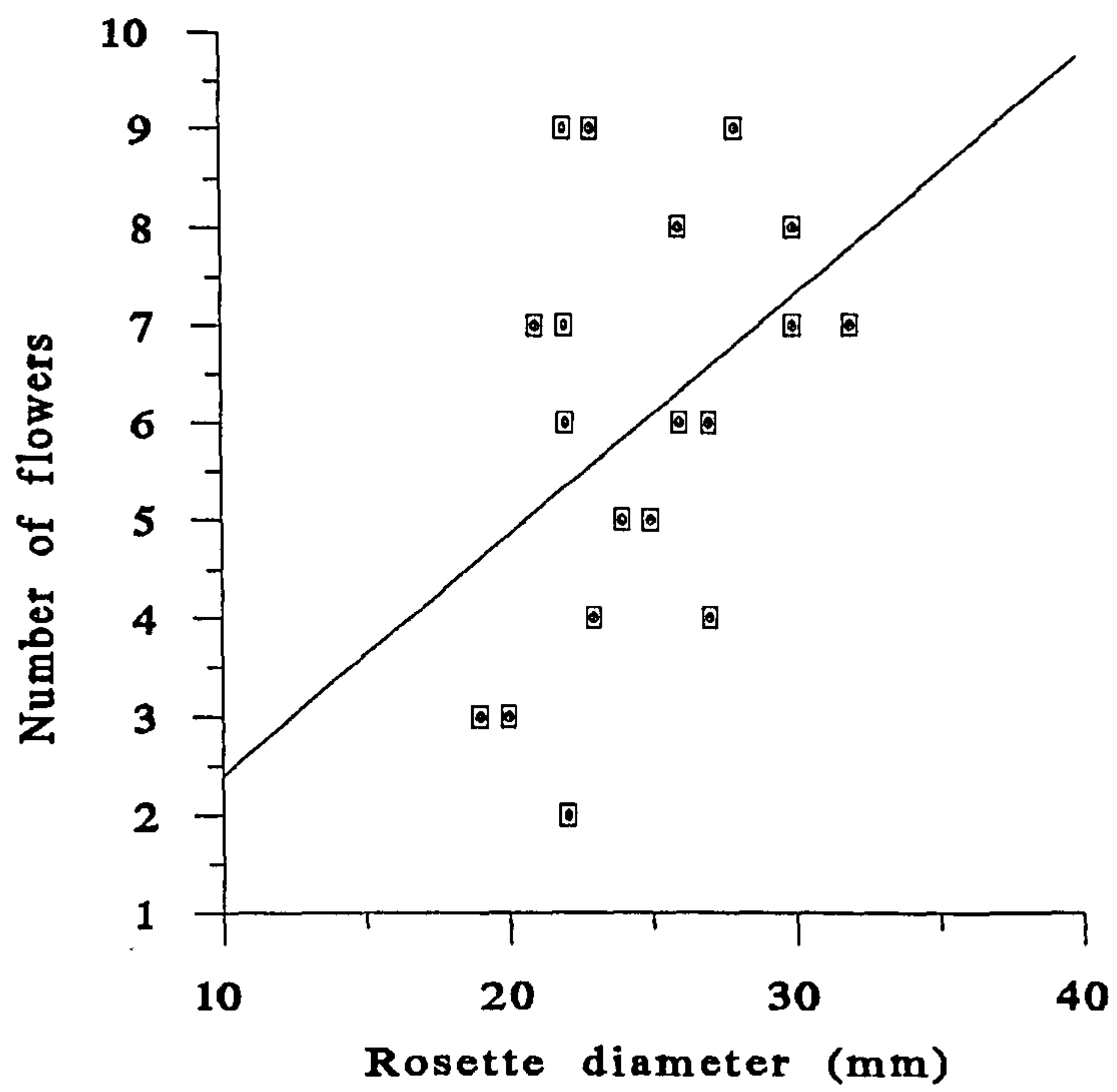


Fig 4 Relationship between rosette size and the number of flowers in the inflorescences of Lychnis alpina at MK1 in 1992.

SEED BANK

The readily germinable portion of the seed bank showed a seasonal variation. It was the highest in the February samples (1002 seeds m⁻², of which 690 were from the 5 cm samples) with identical values in June and October (312 seeds m⁻²). This probably indicates a requirement for vernalisation for Lychnis.

Discussion

There are large differences in the life history traits of Lychnis alpina over its areal distribution. Local climate determines to a large extent the dynamics (demographic characteristics) of the species. Large fluctuations have been observed in its population size in 'alvar' grasslands at Resmo, Öland, Sweden where for example the extremely hot and dry summer of 1992 caused the death of most of the Lychnis there before flowering (H. Prentice, personal communication).

Many studies have considered that the demographic features of a species depend on local conditions and vary between years (e.g. Keddy 1981, Mack & Pyke 1983, Matlack 1987). Matlack (1987) argued that differences in recruitment between nearby sites or within a single site must imply local fine control. There were large differences in the present study in the number of seedling recruits in the two areas although seed production was similar in both 1991 and 1992. Recruitment through seedling establishment in alpine species is only common in open habitats where the soil is not prone to drying out (Marchand & Roach 1980). The oceanic influence is less expressed at Meikle Kilrannoch than in other parts of the Highlands (Brown et al. 1993) and there

are periods without rainfall when soil water deficiency can be limiting, especially in June-July (personal observation). The two areas in the present study differed in soil depth and topographic position. Lychnis seedlings in area 1 with deeper soil had much lower mortality in the summer than those which emerged in area 2. It is likely that the high mortality in the summer was caused by water stress partly owing to the shallower soil there and partly to a more closed vegetation which may have also contributed to a higher exhaustion of soil water. The high mortality in area 1 over the autumn - winter period may have been the result of frost heaving. Area 2 lies in a near-ridge position and therefore any loss of seeds through soil erosion and surface runoff water is unlikely to be replaced. The much lower number of seedlings recorded in area 2 may be a consequence of its topographic position.

Seed dormancy is not predominant in alpine species, however some need after-ripening, vernalisation, or scarification. Relatively high soil temperatures were required for the germination of several species in earlier studies and Bliss (1985) argued that this was probably a selection trait to avoid heavy losses in recruits over winter. The readily germinable portion of the soil seed bank in the present study was highest in February after vernalisation.

Antonovics & Levin (1980) discussed in their review paper the likely advantages for germination of seeds shed in clumps through the possible release of germination stimulators by germinating seeds. The high density patchy distribution of seeds did occur in the present study and seedling density was found very high around mother plants in the early summer following the year of seeding. Inevitably, high seedling mortality was characteristic of these high density seedling patches later. In high contrast, only one seedling emerged out of the 630 seeds sown at 1 cm x 1 cm spacing

in the carrying capacity study (Chapter VI). (Though this result may have reflected some reduced viability of the seeds after two years' storage.) It was not tested experimentally if sowing in clumps versus individually would have affected germination and early establishment in Lychnis.

Vegetative reproduction in Lychnis is a means to rejuvenate its rosettes as the terminal shoot meristem is lost with every flowering and seeding. The survival and future reproductive ability of the genet therefore entirely depends on the production and maintenance of functional apical meristem(s).

The relationship between rosette size and fecundity in Lychnis alpina agreed with reports for other species (Gross 1981, Solbrig et al. 1988,) The lack of correlation between rosette size and the number of flowers in the inflorescences probably means that the observed differences in size were not large enough to account for differences in reproductive allocation and it may in fact be the case that genetic differences are of more importance once Lychnis rosettes reach a threshold size. The cause of the large proportion of unfilled seeds was not investigated in detail. Various reasons including resource limitation have been put forward as possible causes for the abortion of initiated seeds (Fuller et al. 1983, Howe & Westley 1987) Dead whole plant material of Lychnis from MK1 was examined for fungal pathogens but none was found (S.Toth, personal communication). The possible presence of Ustilago violacea, an anther smut commonly infecting Caryophyllaceous species (Ingvarsson & Lundberg 1993, Alexander & Antonovics 1988, Lee 1981, S. Toth personal communication), cannot be ruled out and may cause infertility.

Keddy (1981) emphasized the importance, for studies of the differences in survivorship and reproductive output between sites, of considering, and if possible experimentally

determining, the effects of physical factors and if they act in a density dependent, or density independent manner. Further work on this is required.

There does not seem to be an immediate danger to the survival of the large population of Lychnis alpina at the Meikle Kilrannoch site. However, attention should be paid in future long-term monitoring to genetic variability which may be slowly diminishing by inbreeding at this small site.

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Appendix II

Thesis

2363



Ultramafic Rocks and their Vegetation: an Overview

J. Proctor and L. Nagy

*Department of Biological and Molecular Sciences, University of Stirling, Stirling
FK9 4LA, UK*

Abstract

In this paper the important recent advances in our knowledge of ultramafic rocks and their vegetation are discussed and some major lacunae indicated. The importance of understanding site geology is emphasized and the key role of pedologists – unfortunately poorly represented in this volume – is acknowledged. It is clear that a huge scope remains for descriptive studies – including in-depth site-specific studies – and the need for much more taxonomic research is recognized. Taxonomists are of fundamental importance since they define the units with which ecologists work and their influence pervades all aspects of ultramafic studies. The causes of distinctive ultramafic vegetation are discussed. The widespread influence of magnesium now seems clear and the basis of magnesium tolerance is beginning to be understood. The role of nickel remains enigmatic and apart from the well-documented phenomenon of hyperaccumulation, putative effects of nickel are in need of critical experiments. Other elements such as chromium, cobalt and iron may play a role but much of the early work on them has probably involved analyses of contaminated material and should be disregarded. There has been a number of reports of dramatic responses to field fertilization of ultramafic sites and the importance of low soil nutrient concentrations is assessed. The need for more hydrological studies of ultramafic areas is stressed since there are indications that both drought and waterlogging can exert separate effects. It is acknowledged that all causal factors may interact in a variety of complex ways. Ultramafics offer many intriguing examples of plant evolution some of which have been studied. Several approaches have been tried but progress on understanding evolution is necessarily slow because each species has its own different history. Ultramafic rocks and soils are so diverse that search for an all-encompassing 'serpentine factor' is pointless. The best that can be expected are models which are of value for a limited range of ultramafic situations. The urgent need for conservation of ultramafic areas is as obvious as it is difficult and we add our support to the resolution drafted by the Conference.

Introduction

The papers in this volume and several others recently published elsewhere e.g. for the Balkan Peninsula^{1,2}, California³, Cuba⁴, Italy⁵, Japan^{1,6} and New Caledonia⁷ have emphasized that the study of ultramafic rocks and their vegetation deals not

with a biological curiosity but with an important and under-rated world-wide phenomenon.

It must be admitted at the outset that ultramafic areas are so diverse that the search for an all-encompassing generalization is pointless. The idea of a unifying 'serpentine factor' was revived in 1987 by R. R. Brooks¹ but is not acceptable. Ultramafic rocks are variable but usually contrast chemically and physically with their neighbours and elicit a vegetational contrast which, partly depending on the adjacent rock, may range from the scarcely detectable to the dramatic. Often ultramafics have open stunted vegetation and rare species, but in some cases their vegetation is closed and tall and there may be few or no rarities. The causes of the vegetation are multivariate and interacting and as Slingsby (this volume) has emphasized, can vary greatly within a single site. While admission of these complexities has attracted ridicule⁸ the truth remains that modelling ultramafics is immensely complicated and it would be too optimistic to expect widely applicable models. In spite of their long history of study, ultramafics and their vegetation are in many ways little known. However this volume has demonstrated many recent advances which are here reviewed under the following headings: geology; soils; descriptive studies; the causal factors, calcium and magnesium, nickel and other metals, low nutrients, drought; and plant evolution.

Geology

It is implicit in the account by Coleman and Jove (this volume) that full attention to site geology is important, since this may largely explain the vegetation. For example, ultramafic rocks with relatively high proportions of calcareous minerals such as pyroxenes will be less likely to show a distinctive vegetation since, as is amplified later, calcium ameliorates the effects of magnesium and other potential toxins. Calcium-rich travertines can be of local importance for plant distribution⁹ and can still issue from ultramafic rocks during present-day hydrothermal alteration (serpentinization) (Coleman and Jove, this volume).

Serpentinization is associated with textural changes in the ultramafic rocks. Malpas¹⁰ recognizes three categories of serpentinite on the basis of their physical appearance: blocky or massive serpentinite which undergoes little or no deformation during serpentinization; sheared serpentinite which is developed by a progressive deformation; and cross-fibre serpentinite which develops in veins that vary from microscopic to macroscopic. On Unst, Shetland, much of the ultramafic area is of limited interest ecologically and has blocky serpentinites^{11,12}. On the hill called the Keen of Hamar, serpentinization has apparently resulted in cross-fibre dunite which weathers more readily and gives rise to a shallow coarse-textured soil which harbours an outstanding flora. In this case the microscopic cross-fibres have been weathered away, leaving the variously serpentinized and fragmenting bedrock as the soil parent material. This is a good example where a geological difference within an ultramafic mass is of greatest importance. Similar contrasts occur elsewhere: for example, compare the Swedish sites at Kittelfjäll (where the soil surface bears a marked resemblance to that of the Keen of Hamar) with the massive ultramafic outcrops at Junsterklumpen¹³.

We should beware of circular arguments in interpreting geology from vegetation and accept with caution the lore of the geobotanical prospector. In the Far East, small-stature forest is often said to occur on ultramafic rock (Whitmore¹⁴ has described 'rain forest on ultrabasic rock' as a distinct formation on the basis of vegetation physiognomy) and there is a temptation to deduce the underlying geology from the vegetation. This may lead to erroneous interpretations. Large stature rain forest can grow on ultramafic rocks e.g. on Gunung Silam, Sabah, and the occurrence of small-stature forest at higher altitudes there is related to the presence of a cloud cap and not to the underlying geology (Ref. 15 and L. A. Bruijnzeel *et al.*, unpublished). Borhidi (this volume) has described a compression of vegetation zones on Cuban ultramafic mountains which has a different (but unknown) explanation from that on Gunung Silam. An example from the Philippines (Fig. 1) shows large-stature forest on serpentinite at the bottom of a valley near a geological boundary. Higher up the serpentinite slope the 'forest' height fell to 2 m but the facile assumption that such stunted vegetation coincided with the geological boundary was unfounded and as is discussed later, the causes of this stunting seem to be connected with the site's hydrology (L. A. Bruijnzeel *et al.*, unpublished). A further example concerns the famous low-stature forest at about 2500 m on Mount Kinabalu, Sabah. This is commonly regarded as indicating an ultramafic bedrock and yet the only published soil analyses¹⁶ from this forest suggest no ultramafic influence.

Soils

A contemplation of the huge variety of ultramafic soils worldwide¹⁷ or even within a single region (e.g. Alexander *et al.*¹⁸ for California) convinces one that there is no unifying 'serpentine factor'. However, whatever their variety, the ultramafic soils are always a major cause of the vegetation they bear and their thorough study should have the highest priority. It is unfortunate that the Conference has had no contribution from pedologists but some principles of the formation of ultramafic soils were given by Coleman and Jove (this volume).

There is a classic instance in Britain, on the Lizard Peninsula, where the studies of parent material¹⁹ have solved difficult ecological problems. Carter *et al.*¹² on the Keen of Hamar and Proctor *et al.*²⁰ for Meikle Kilrannoch have shown the importance of an ultramafic bedrock versus an ultramafic drift as a parent material.

Satisfactory simulation of the ultramafic soil environment is a prerequisite for critical experimental studies. Johnston and Proctor²¹, Proctor *et al.*²², and Vergnano Gambi *et al.*²³ have attempted this in a preliminary way but it is appreciated that soil solution chemistry is complex²⁴. Conventional soil solution extracts are made when the soil is very wet and may convey an erroneous impression of innocuousness since the solution may become more toxic to plants as it dries out. Techniques for examining solutions in drier soils are being developed and Hinkley²⁵ for example, working on non-ultramafic soils, used an isotopic technique that permitted the investigation of small volumes of soil solution. He showed approximately ten-fold increases in soil solution potassium concentrations and five-fold increases in calcium concentrations in a naturally drying soil. There is clearly a possibility of enhanced

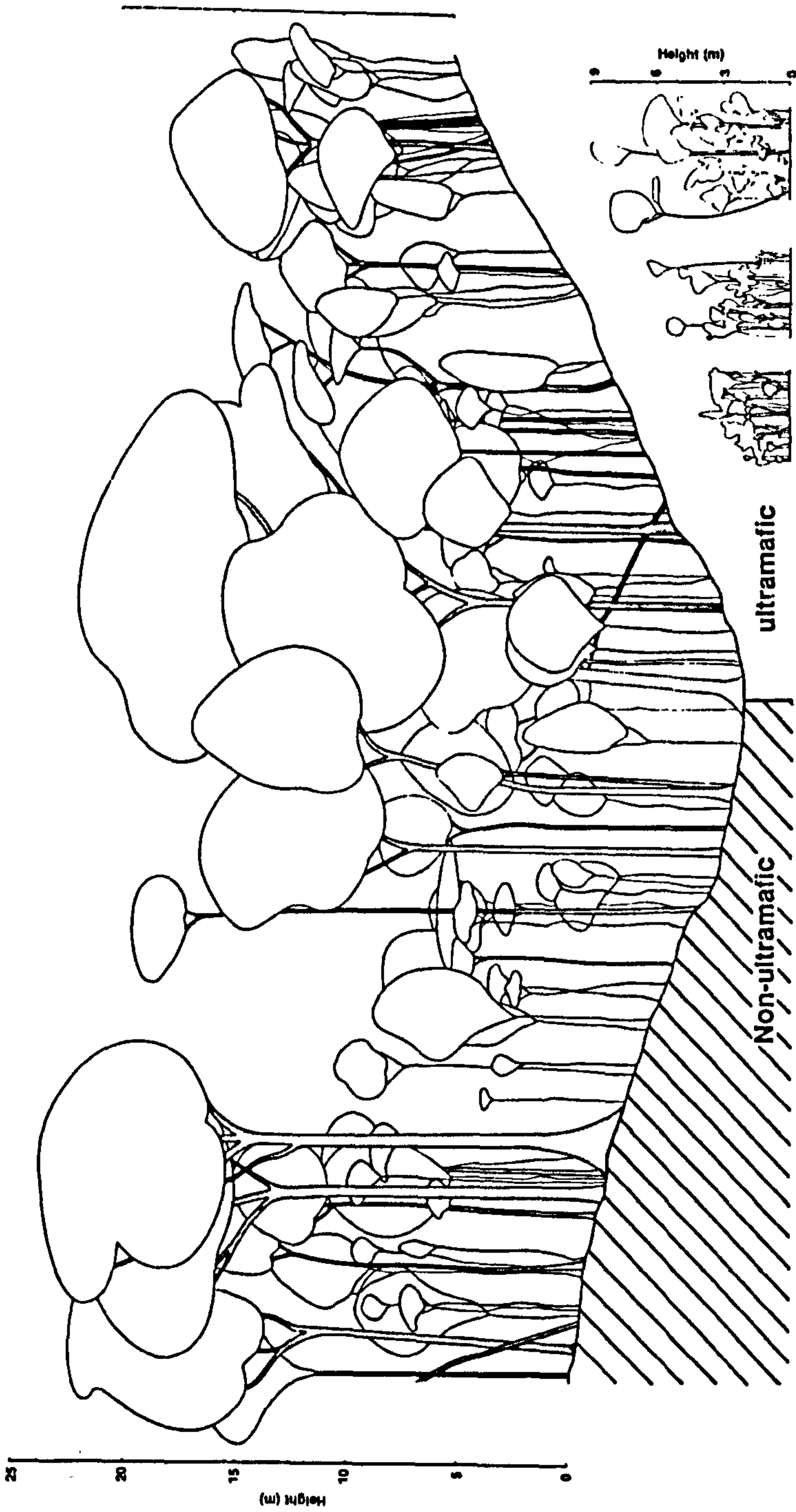


Figure 1 Lowland evergreen rain forest at the junction of non-ultramafic (greywacke) and ultramafic rocks on the island of Palawan, Republic of the Philippines (from L. A. Bruijnzeel *et al.*, unpublished). The stunted forest (inset) is situated about 120 m above the junction, on very shallow ultramafic soils.

toxicities in drying ultramafic soils. A further possibility is that of enhanced ionic toxicities in frozen soils which may interact with frost-heaving effects. An alternative view (Robertson, this volume), which also needs further investigation, is that ultramafic soils are more toxic when they are wet. This is supported on theoretical grounds by Jenne²⁶ who has suggested that nickel is likely to be brought into solution at low redox potentials.

The most dynamic soil processes and certainly those most relevant for plant establishment will occur in the upper 5 cm of soil. This zone is rarely considered separately and most workers have sampled to greater depths, mixing horizons and possibly confounding subtle and crucial soil differences as they did so.

Vegetation description

Without denying the importance of experiments, the descriptive approach is still a necessity and important papers are still emerging. For example, interest in South Africa has been renewed by Cole²⁷ and Morrey *et al.*²⁸; Australia now has accounts for the west²⁹ and work has at last started in the east (e.g. ref. 30, and this volume); accounts are emerging from Brazil (Ref. 31, and Brooks *et al.*, this volume); and descriptions for the Philippines are in preparation (A. J. M. Baker, L. A. Bruijnzeel and J. Proctor). It must be said however that tropical ultramafic vegetation is still poorly documented including some very large areas (up to 8000 km² contiguously) in Indonesia¹⁶. Some important ultramafics elsewhere seem virtually unknown biologically and we might mention those of China, Iran, Mongolia and Oman in this respect. As well as descriptions of new areas we need more intensive investigations of well-known examples. The more we look at ultramafic areas the more each one is likely to reveal. D. R. Slingsby's study^{12,32} of the small area of the Keen of Hamar in Shetland is one example. Armstrong and Huenneke (this volume) provide another, from Jasper Ridge in California. These and other studies offer an approach to understanding the influence of physiographic factors such as slope, aspect and elevation, and disturbance factors such as fire, grazing and burrowing animal activity on ultramafic community structure and diversity. Long-term site-specific studies are a prerequisite for underpinning the adequate conservation of ultramafic areas.

Calcium and magnesium

Calcium deficiency *per se* was emphasized as an important factor in ultramafics by Kruckeberg³³ and Walker³⁴. More recent work, e.g. Ref. 35, has suggested that calcium deficiencies are unlikely and this factor is now much less widely discussed than formerly. Nevertheless, Proctor *et al.*²² drew attention to very low (< 0.8 mg l⁻¹) calcium concentrations in some Scottish ultramafic soils and it seems possible that calcium deficiency may be reconsidered when the precise toxic actions of other ions are known at the molecular level. A. R. Kruckeberg (personal communication) now (1992) regards calcium deficiency as an 'enigma'. What is certain is that any

consideration of calcium deficiency in ultramafics will always involve other ions, particularly magnesium, with which calcium interacts.

There is no doubt that in many ultramafics magnesium has an important influence. It has three distinct roles. First, by occupying a high proportion of the exchange sites it incidentally raises the soil pH. This alone is enough to cause a distinctive flora in open areas surrounded by acid soils¹¹. Secondly it is toxic, particularly when calcium is at relatively low concentrations. Many ultramafic plants are tolerant of high magnesium concentrations^{34,36}. The three cases we know of ultramafic soils (Clear Creek in California, Kitefjäll in Sweden, and Meikle Kilrannoch in Scotland), which are so toxic that they rapidly kill seedlings of non-tolerant plants, have all involved acute magnesium toxicity (Refs. 37, 38 and J. Proctor, unpublished). The toxicity of magnesium depends not only on the proportion of calcium present in solutions but also on the actual concentrations of both elements³⁶. Calcium is relatively more effective at ameliorating magnesium toxicity when the actual concentrations of both ions are low. This may explain those reports of soils with high quotients of exchangeable Mg/Ca but with no ultramafic effect on the vegetation (e.g. Ref. 39 for soils under a montane rain forest in Jamaica). Some of these instances deserve further investigation however. For example D. R. Morrey (personal communication) has found non-ultramafic South African soils of high pH and with a Mg/Ca quotient of 10 and which show no suggestion of an ultramafic effect on the vegetation. So readily is magnesium toxicity ameliorated by calcium that, as we commented earlier, the vegetation of calcareous ultramafics invariably contrasts with those where calcium is in short supply^{40,41}. Thirdly, magnesium has complex inter-relationships with other elements. It can ameliorate nickel toxicity⁴² and has been shown to reduce plant nickel concentrations, probably in a complex interaction in which calcium is involved⁴³, or exacerbate it²¹. It has long been known to have an antagonistic effect on the uptake of potassium⁴⁴ and this is briefly discussed later.

Plant analyses have revealed huge differences in magnesium and calcium concentrations between species growing on similar sites (e.g. Refs. 41, 45) variously interpreted as involving exclusion or accumulation of one or both elements. There are some reports of impressive magnesium accumulations. Roberts⁹ found up to 150,000 $\mu\text{g g}^{-1}$ magnesium in the dry matter of some members of the Caryophyllaceae in Newfoundland.

There is evidence that many ultramafic plants may have a relatively high requirement for magnesium (Goodwin-Bailey *et al.*, this volume, and Refs. 46-48) although Kinzel⁴⁹ has doubted if this applies to magnesium concentrations within the cell.

In spite of an important role of magnesium in many ultramafics and the obvious wide range of species adaptations to it, we know so little about the mechanisms of their resistance or tolerance and of the precise toxic action of magnesium in non-resistant species. This is perhaps the greatest gap in our knowledge of ultramafic plants.

A little information is available on tolerance. Some plants bind magnesium in the vacuole as magnesium oxalate (e.g. *Tunica saxifraga* in the Caryophyllaceae)⁵⁰ whilst others such as *Sedum album* (Crassulaceae) and *Biscutella laevigata* (Brassicaceae) maintain high vacuolar concentrations of magnesium⁵¹. Tibbetts and Smith (this volume) have shown that half the vacuolar magnesium in an ultramafic

race of *Sedum anglicum* was in the form of soluble chelates (citrates, isocitrates and malates) whilst the other half was as Mg^{2+} ions. It seems likely that there are magnesiophobic species and magnesiophilic species in the sense that analogous terms are used for calcium. Kinzel⁴⁹ has discussed how species of Caryophyllaceae may accumulate solid magnesium oxalate along with calcium oxalate in the vacuole although because of the solubility products of the components concerned this could lead to a vacuolar solution Mg/Ca quotient of nearly 50,000.

More data are needed on the proportions of dissolved and undissolved magnesium and its compartmentation within the cell; the means by which some plants take up proportionately more and others proportionately less magnesium than calcium; and the means by which magnesium poisons non-tolerant plants.

Nickel

The presence of higher-than-normal quantities of nickel is one of the most general features of ultramafic soils. However the role of this element in causing the unusual vegetation is not clear and its influence undoubtedly varies from area to area. Many assumptions about causal roles for this element in ultramafic vegetation are unfounded and in-depth studies have often eliminated the element as an important factor. For example, Kruckeberg³ has commented that (for cobalt, chromium and iron as well as nickel): "there has been no decisive evidence that shows any of these elements affects plant growth in western North America". In New Zealand, Lee⁵² in a review of all ultramafic areas, comments that only in soils from ultramafics in the south-west of the South Island is nickel likely to reduce plant growth critically. Carter *et al.*¹² could find no evidence that nickel was a cause of the barrenness of the ultramafic Keen of Hamar, Shetland.

Nickel as an essential element

The discovery in 1975 that nickel is a component of the enzyme urease^{53,54} led to renewed research concerning the role of nickel in higher plants. Urease is found in many higher plant species and also in micro-organisms. When urea was used as a sole nitrogen source, plant growth depended on the nickel-containing urease activity and the addition of chelators decreased nickel availability, which in turn resulted in decreased plant growth^{55,56} and visual symptoms developed in the form of necrotic lesions at the leaf tips where urea accumulated. However nickel was not necessary for plants when the nitrogen source was ammonium ions^{55,56}. The need for nickel for optimal cereal growth was shown by Brown *et al.*⁵⁷, who found that barley, oat and wheat plants raised without nickel accumulated up to 20 times higher levels of urea in their leaves, and barley produced about one third less root and shoot mass without nickel.

The true essentiality of nickel as a micronutrient for higher plants has now been proved by Brown *et al.*⁵⁸. They found that barley plants fail to complete their life

cycle in the absence of nickel and that the addition of nickel to the growth medium completely alleviated deficiency symptoms. Apart from its importance in urease activity it appears that the metabolic role of nickel is unknown. Brown *et al.*⁵⁸ believe that it may have essential functions in seed maturation – possibly involving the transport of nutrients, and in the movement of iron to plant cells. Relationships between plant iron and nickel have long been recorded and Table 1 shows an instance where nickel appears (it may be artefactual, as discussed later) to cause enhanced uptake of iron by roots, particularly at lower magnesium concentrations.

Table 1

Concentrations of calcium, magnesium, iron and nickel in the shoots and roots of *Avena sativa* L. grown with (1.25 mg l⁻¹) and without nickel at a range of concentrations of magnesium in water culture. Values in parentheses refer to the without nickel treatments; no plant nickel analyses were made for the without nickel treatment (Data of J. Proctor, unpublished).

Solution Mg (mM)	Ca	Mg ($\mu\text{g g}^{-1}$ dry matter)	Fe	Ni
		Shoot		
1.25	2050 (1900)	3090 (3700)	184 (208)	172
2.5	1320 (1060)	4830 (4700)	126 (155)	133
5.0	807 (619)	6270 (5730)	125 (126)	107
7.5	687 (729)	7460 (6100)	114 (195)	91
		Root		
1.25	1060 (772)	4360 (5120)	2560 (905)	2530
2.5	732 (696)	3670 (6730)	3190 (950)	1700
5.0	581 (495)	3700 (7090)	1880 (1430)	1240
7.5	595 (573)	5460 (8110)	2550 (2240)	962

The quantities required by the crop plants so far investigated appear to be very low (of the order of 0.1 $\mu\text{g g}^{-1}$, or less, of tissue dry matter) however. Ultramafic plants might need more nickel and a quantification of their requirements would be revealing. It is not too fanciful to imagine that the emphasis may shift from putative toxin to putative micronutrient for some plants in ultramafic soils. There are some indications already that nickel may have a positive effect on ultramafic plants. Gabbrielli *et al.*⁵⁹ have shown that root-surface phosphatase from *Alyssum bertolonii* is stimulated by Ni²⁺ concentrations between 0.001 and 0.01 mM. Vergnano Gambi *et al.*⁶⁰ reported that nickel at 0.25 mM has a marked stimulatory effect on the germination of *A. bertolonii*.

Nickel in plant tissues

There has been an explosion of work on nickel-accumulating species (Baker *et al.*, this volume, and Reeves, this volume, are recent examples) which is justified because nickel accumulation is one of the most remarkable of all biological phenomena. Among the more recent discoveries is that nickel accumulators have

been found in more northerly areas: e.g. for *Thlaspi caerulescens* on an ultramafic site in Ayrshire, Scotland (A. J. M. Baker, personal communication) [perhaps the only report for a formerly glaciated site], Newfoundland⁹ (on former nunataks), and several more (in addition to, but to less extreme than *Alyssum bertolonii*) in Italy⁵. The transient nature of nickel accumulation which O. Vergnano Gambi (personal communication) has observed in some alpine species, is an aspect of the phenomenon which deserves further work. The evidence for nickel accumulation from low nickel soils is very limited^{61,62} and should be further investigated. The putative threshold of nickel concentrations between normal and hyperaccumulator species does not occur in Brazil (Reeves, this volume, and Brooks *et al.*, this volume) and may be an artefact of sample selection elsewhere. There has been clarification of the biochemistry of nickel accumulation¹. Still and Williams⁶³ proposed a mechanism for the selectivity of nickel hyperaccumulators which involves highly selective ligands with two or more nitrogen donor atoms. Boyd and Martens (this volume) have pointed a way at last towards the understanding of the ecology of nickel accumulation. There is evidence from their work on *Streptanthus polygaloides* that the element protects against herbivores and pathogens. Schlegel *et al.* (this volume) have shown that nickel from accumulators can affect soil bacteria.

Fascinating though the research on accumulation has been, it has added little to our understanding of the more general roles of this element in the vegetation and it must be admitted that nickel hyperaccumulators account for only a small proportion of any flora in which they occur. As for magnesium, the way in which plants seem to regulate the uptake and translocation of nickel needs elucidation. Root systems must often play a key role. Table 1 shows that in oats (not nickel tolerant), nickel concentrations were much higher in the roots than shoots. Wiltshire⁶⁴ showed that *Dicoma niccolifera* had substantial nickel concentrations in its roots. Menezes de Sequeira and Pinto da Silva⁶⁵ in their analyses of Portuguese ultramafic plants showed that roots had more nickel than leaves in several species, most spectacularly in *Asplenium cuneifolium* where the roots had 930 $\mu\text{g g}^{-1}$ nickel and the leaves 30 $\mu\text{g g}^{-1}$. Some of the plants examined by Menezes de Sequeira and Pinto da Silva⁶⁵ had low concentrations of nickel in both roots and shoots and the question of exclusion of this element should be carefully examined.

Nickel toxicity

The question of nickel toxicity has many facets and this was discussed by Proctor and Woodell¹⁷. Putative nickel toxicity has only rarely been confirmed with sufficiently critical experiments. The complexity of the soil supply of nickel was well discussed by Jenne²⁶. He showed the important effect of hydrous oxides of iron and manganese on nickel availability and emphasized the crucial roles of pH and redox potential in determining the availability (and efficacy) of these hydrous oxides. In well-drained soils with a pH greater than 6.0, nickel seems unlikely to reach toxic concentrations. However, there may be local or short-term reductions of redox potential (as envisaged by Robertson, this volume) or localized decreases of pH near root surfaces⁶⁶ which might result in nickel being available at higher concentrations to plants.

Nickel toxicity is clearly dependent on calcium, magnesium and other ions^{43,67}. High magnesium concentrations can exacerbate nickel toxicity²¹ but in the case shown in Table 1 magnesium ameliorated the nickel toxicity symptoms and reduced the tissue concentrations of nickel⁴². The relationship between iron and nickel suggested in Table 1 has long been emphasized (e.g. Ref. 68). Chaney *et al.*⁶⁹ raised the question that former hydroponic test cultures for indicating the effects of cadmium, copper, nickel and zinc may have been confounded by the displacement of iron by the above metals from the Fe-chelate (e.g. the widely used NaFe-EDTA) used to supply iron for plants growing in the culture solution. Bearing this in mind, we subjected some of the culture solutions used in earlier experiments to an analysis for ionic balances using the GEOCHEM PC computer program^{70,71}. The results for the solution used by Proctor and McGowan⁴² showed that as a result of displacement of iron by nickel from EDTA, chelated iron was only 71.5% as compared with the non-nickel treatment. Only 1.4% of the Ni²⁺ (3.01×10^{-7} M) was present in the solution as a free ion. The plants certainly took up nickel (Table 1), which showed a clear relationship with increasing solution magnesium, but it is not known if the effects of nickel in increasing root iron concentrations reflect a genuine metabolic relationship between these two elements (and perhaps magnesium) or is a result of iron displacement from EDTA. In the work of Johnston and Proctor²¹, where iron was supplied at much lower concentration (10^{-5} M), only 4.4% of the iron remained chelated to the EDTA and nickel replaced the rest of it. Fifty-eight percent of the nickel was present in the solution in free ionic form (1.41×10^{-5} M). Again nickel uptake by plants occurred and so did iron uptake despite the latter's much lower availability from Fe-EDTA. Grasses (*Avena sativa* and *Festuca rubra*) were used in these experiments which might explain the small differences in their iron uptake under widely differing Fe-EDTA iron supply. As is known, these species use the highly efficient Strategy II⁷² to gain iron when it is in short supply. The work of Johnston and Proctor²¹ (Fig. 2) shows the influence of micronutrients on nickel toxicity. Only when micronutrients (boron, copper, manganese, molybdenum and zinc) were reduced to low concentrations were plant symptoms (of magnesium and nickel toxicity simultaneously), matching those in the soil, obtained. Similar experiments should be made, using the recent advances in controlling ionic concentrations in culture solutions, to identify precisely the micronutrients involved.

Nickel resistance and tolerance

One must be prepared for unexpected results. On the Keen of Hamar a nickel-rich area occurs¹² which bears a vegetation of widespread species. Is the adaptation to nickel toxicity as easily achieved as this observation suggests? In that case can nickel ever be said to have a determining influence on vegetation? There is further evidence of an adaptation to high nickel in vegetation where the element was having no obvious effect. Analyses of fresh leaves and litterfall from Gunung Silam, Sabah⁷³ showed substantial resorption of nitrogen and phosphorus but an increase in nickel before the leaves fell – suggesting a possible excretion mechanism for the element.

There is the problem of the relevance of tolerance tests. For example Proctor³⁶ found ample evidence of nickel tolerance in grasses from British ultramafic sites

where subsequent work has revealed no clear influence of this element^{12,74,75}. Is this tolerance adaptive in a subtle way? Is it non-specific tolerance related to magnesium tolerance? Certainly nickel and magnesium tolerance can be highly correlated⁷⁶ but the correlation is not perfect and it is possible to get nickel tolerance without magnesium tolerance as Proctor³⁶ showed for *Agrostis vinealis* from Rhum. Borhidi (this volume) referred to Cuban nickel-soil plants which will not grow in high magnesium soils but high-magnesium soil plants will grow in high-nickel soils. Schat (this volume) has given an insight into how tolerance mechanisms may be present but not adaptive. Bannister and Woodman (this volume) have shown how tolerance may be a reflection of low growth rate. It now seems that demonstrations of nickel tolerance in sites such as the Keen of Hamar and Rhum cannot be used as conclusive evidence that nickel has an important influence there.

Finally, the substantial advances which have been made in the understanding of nickel resistance in bacteria must be mentioned. In an *Alcaligenes eutrophus* strain CH34 (from a nickel-polluted site) the nickel (and cobalt) resistance is determined by a plasmid (pMOL 28) which is transmissible to other bacteria. The nickel resistance has been shown to be an inducible property⁷⁷ and is due to an energy-dependent specific efflux^{78,79}. Highly nickel-resistant bacteria have now been isolated from ultramafic soils from Australia, New Caledonia, and Scotland (Ref. 80 and Schlegel *et al.*, this volume). The most nickel-resistant were isolated from organic soils under nickel-accumulating shrubs and trees in New Caledonia. Some of these bacteria could tolerate up to 280 $\mu\text{g g}^{-1}$ nickel in a culture medium. Schlegel *et al.*⁸⁰ consider it likely that all the bacteria in nickeliferous soils will be tolerant and that there is an active nickel cycle in nature. It is likely that important breakthroughs will be made by these bacterial studies when they are applied to many more sites. We must add the cautionary notes however that the field concentrations of nickel are difficult to relate to those in culture media which have a gamut of substances capable of modifying the effects of this element. Furthermore the bacterial work has so far not considered the possibility that nonspecific magnesium resistance might be involved in the nickel resistance of bacteria of ultramafic soils.

Other possibly toxic elements

There is relatively little evidence beyond plant analyses that chromium is involved in a major causal way in ultramafic soil-plant relations¹⁷. Brooks and Yang⁸¹ have shown that some earlier plant analyses seem to have overestimated tissue chromium because of an unawareness of the ease with which this element can contaminate plant material from soil particles. Perhaps the most convincing recent evidence for a role for chromium is that of Morrey *et al.*²⁸ who analyzed several South African plant species and found two which absorb and translocate chromium. *Sporobolus pectinatus* had 334 ± 99 (SE) $\mu\text{g g}^{-1}$ Cr in its roots and 82 ± 22 $\mu\text{g g}^{-1}$ in its leaves; *Sutera* sp. had 60 ± 10 $\mu\text{g g}^{-1}$ in its roots and 248 ± 52 $\mu\text{g g}^{-1}$ in its leaves.

The curious case of the New Caledonian epiphytic moss *Aerobryopsis longissima*⁸² must also be mentioned. This species was shown to contain 200–700 $\mu\text{g g}^{-1}$ dry weight of chromium and since it was collected about 2 m above the ground

it is safe to assume that there was no soil contamination. Its host plants – which included the nickel hyperaccumulator *Homalium guillainii* – invariably had much lower chromium concentrations.

Cobalt is another element which is possibly important in ultramafics but apart from some high values in a few plant analyses there is little to suggest a major role. An extraordinary accumulation of cobalt was observed by Reeves and Baker⁶³ in *Thlaspi goesingense* (from Bernstein, Austria) grown on a soil which was collected from New Caledonia. The plants (with an ultramafic origin) of this species had a mean cobalt concentration of 1790 $\mu\text{g g}^{-1}$ – much higher than the highest value (450 $\mu\text{g g}^{-1}$ for a sample of *Phyllanthus serpentinus*) recorded for New Caledonian plants collected in the field in that country⁷.

There have been several accounts of high tissue iron concentrations following the demonstration by Ritter-Studnička and Dursun-Grom⁸³ of 13,700 $\mu\text{g g}^{-1}$ in the leaf dry matter of *Potentilla tomassiniana* from Yugoslavia. The highest value so far recorded is 44,000 $\mu\text{g g}^{-1}$ for *Arenaria humifusa* and *A. marcescens* (Caryophyllaceae) in Newfoundland⁹. Even more so than chromium, iron is likely to be a contaminant in plant analyses. It is probably impossible to wash out of naturally grown plant material and without careful experimental work we may never be sure of its true role in metabolism. As we discussed earlier, several authors (e.g. Crooke *et al.*⁶⁸ and Mizuno and Nosaka⁶) have commented on experiments which have shown that high quotients of iron/nickel are associated with decreasing nickel toxicity symptoms in crop plants. Table 1 shows that higher root Fe/Ni quotients are associated with decreased nickel toxicity symptoms in oats.

Manganese accumulation has been reported for leaves in a large number of New Caledonian species⁷. Twenty-one percent of the species analyzed by Jaffré had more than 1000 $\mu\text{g g}^{-1}$ manganese. Nine species had more than 10,000 $\mu\text{g g}^{-1}$ with the highest value of 55,000 $\mu\text{g g}^{-1}$ in *Macadamia neurophylla* (Asteraceae). A few instances of plants with more than 1000 $\mu\text{g g}^{-1}$ manganese in foliar dry matter were recorded by Proctor *et al.*⁷³ on Gunung Silam where the highest value was 13,700 $\mu\text{g g}^{-1}$ in an unidentified *Eugenia* species (Myrtaceae). Accumulations of manganese are of interest in view of the report by Williams⁸⁴ of an exacerbation of nickel toxicity symptoms by manganese in oat plants grown in an Australian ultramafic soil. The plants had normal iron status, and Williams envisaged an interaction between manganese and iron within the plant which effectively decreased the Fe/Ni quotient. Work on thirty-one species of *Alyxia* from New Caledonia⁸⁵ revealed several species with foliar manganese concentrations in excess of 1000 $\mu\text{g g}^{-1}$ and correlations with other foliar elements led the authors to suggest that manganese might substitute for calcium or potassium, or both in some of their metabolic roles.

Accumulations of iron and manganese by ultramafic plants are surprising in view of the relatively high pHs and redox states which often prevail in the soils.

Low nutrients

Low nutrients are likely to be a key feature in maintaining the character of the ultramafic vegetation. For example in Scotland on Rhum and the Keen of Hamar,

nutrient addition experiments have provided spectacular results in increasing plant cover and changing the species composition. In Santa Clara, California, nutrient addition has allowed, without further disturbance, the invasion of ultramafic grassland (which is a refuge for native species) by alien species from the surrounding non-ultramafic areas⁸⁶. In all these cases fertilization has caused an increase in biomass. On Rhum, fertilization resulted in a marked increase in species richness; on the Keen of Hamar and in Santa Clara there was a decrease in species richness. Field nutrient addition experiments can usefully be extended and they certainly point to the dangers of eutrophication of ultramafic sites where conservation is of concern. Their interpretation however is often not straightforward⁸⁷ and generalizations such as those just seen for species richness may be difficult to apply. The influence of nitrogen source on ultramafic plants needs further investigation, particularly in view of the demonstrations of the likely importance of ammonium versus nitrate nutrition in accounting for differences between the vegetation on acidic and calcareous soils^{88,89}. Wiltshire⁹⁰ examined the effect of nitrogen source on nickel uptake by non-resistant crops on Zimbabwean ultramafics. He found that nickel uptake was less with ammonium as the nitrogen source in low-nickel soils but higher with the same source in high-nickel soils. Preliminary work has been done on *Agrostis vinealis* from the Scottish Meikle Kilrannoch site, where nitrate is the predominant anion in the soil solution. The results (J. Proctor and E. J. Brown, unpublished) were complex but revealed a substantially higher relative growth rate of roots in simulated soil solutions with nitrate rather than ammonium as the nitrogen source. Additions of nitrate in any form to the Meikle Kilrannoch soil usually cause enhanced toxicity – we believe this is because it causes excess magnesium to be brought into solution (although Kinzel⁴⁹ offers a different explanation).

In some cases ultramafic plants may be confined to their substratum because they are unable to obtain major nutrients from the surrounding soils. This appears to be the case for *Lychnis alpina* at Meikle Kilrannoch, Scotland. Here the ultramafic site is surrounded by acid highly organic soils (blanket peat). *Lychnis* does not occur in these areas and a glasshouse experiment was made (J. Proctor and E. J. Brown, unpublished) in which various fertilizers were added to the ultramafic and to the peat soil. The only treatment which enabled the *Lychnis* on peat to equal its growth on the ultramafic soil was +CaNPK in which the pH was raised (by the CaCO₃ addition) as well as major nutrients added (Fig. 2). This experiment demonstrates an instance where an ultramafic species is apparently unable to obtain its major nutrients from surrounding soils and shows the information that may be gained if 'control' soils in experimental work are not garden soils, but non-ultramafic soils adjacent to the ultramafic soils in the field. Ultramafic plants probably have a general well-developed ability to obtain nutrients from their imbalanced chemical environment. Their ability in calcium uptake from high magnesium solutions has been frequently demonstrated experimentally by workers in the western USA³. There is evidence from Madhok and Walker⁴⁶ that the ultramafic *Helianthus bolanderi* can maintain higher plant-potassium concentrations at high solution magnesium concentrations than can non-ultramafic *H. annuus*. Extraordinarily high potassium concentrations (up to 9% dry matter) have been reported in some ultramafic plants from Greece⁹¹ and up to 9.4% from Meikle Kilrannoch in Scotland⁴¹ and may perhaps reflect the storage of an element in short supply.

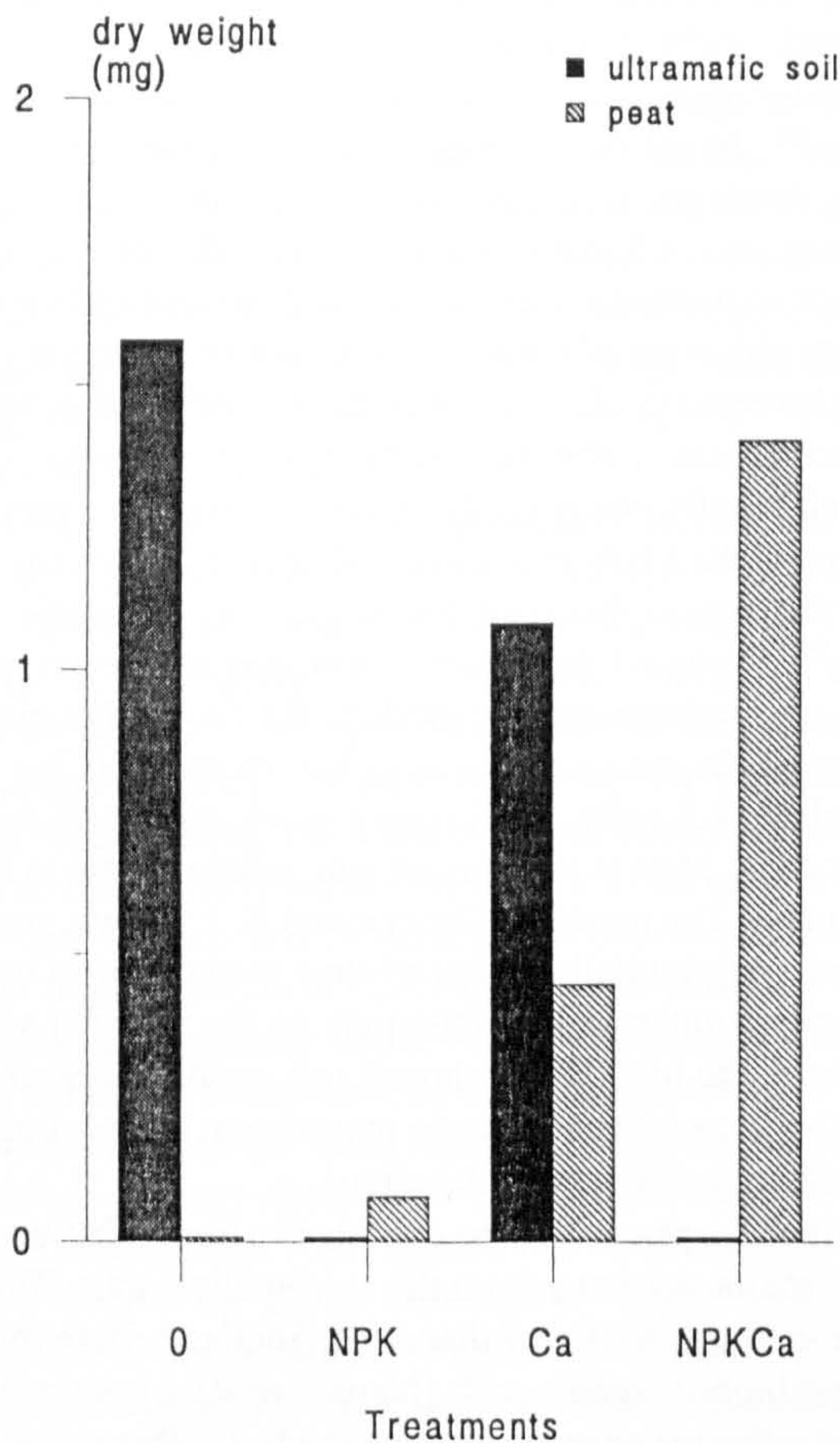


Figure 2 The growth of *Lychnis alpina* in unaltered non-ultramafic (peat) soil and ultramafic soil, and in the same soils to which major nutrients, calcium, and together major nutrients and calcium had been added. The soils were collected within 25 m of the geological boundary at Meikle Kilrannoch, Scotland (J. Proctor and E. J. Brown, unpublished).

The possibility that metals, including magnesium and nickel, may reduce the supply of phosphorus by the soil or render it insoluble within the plant is often mentioned but little investigated. There is much scope for further work within this area and we are mindful of Jeffrey's⁹² striking negative correlation (involving non-ultramafic soils) between soil lead and the supply of phosphorus in his work on the distribution of the rarity *Kobresia simpliciuscula* (Cyperaceae).

The older literature on the role of micronutrients has been reviewed by Proctor and Woodell¹⁷. Figure 3 shows the influence of micronutrients (although no individual micronutrient was identified) on magnesium and nickel toxicity in *Festuca rubra* and it is clear that any experimental work on ultramafic plants must take this effect into account.

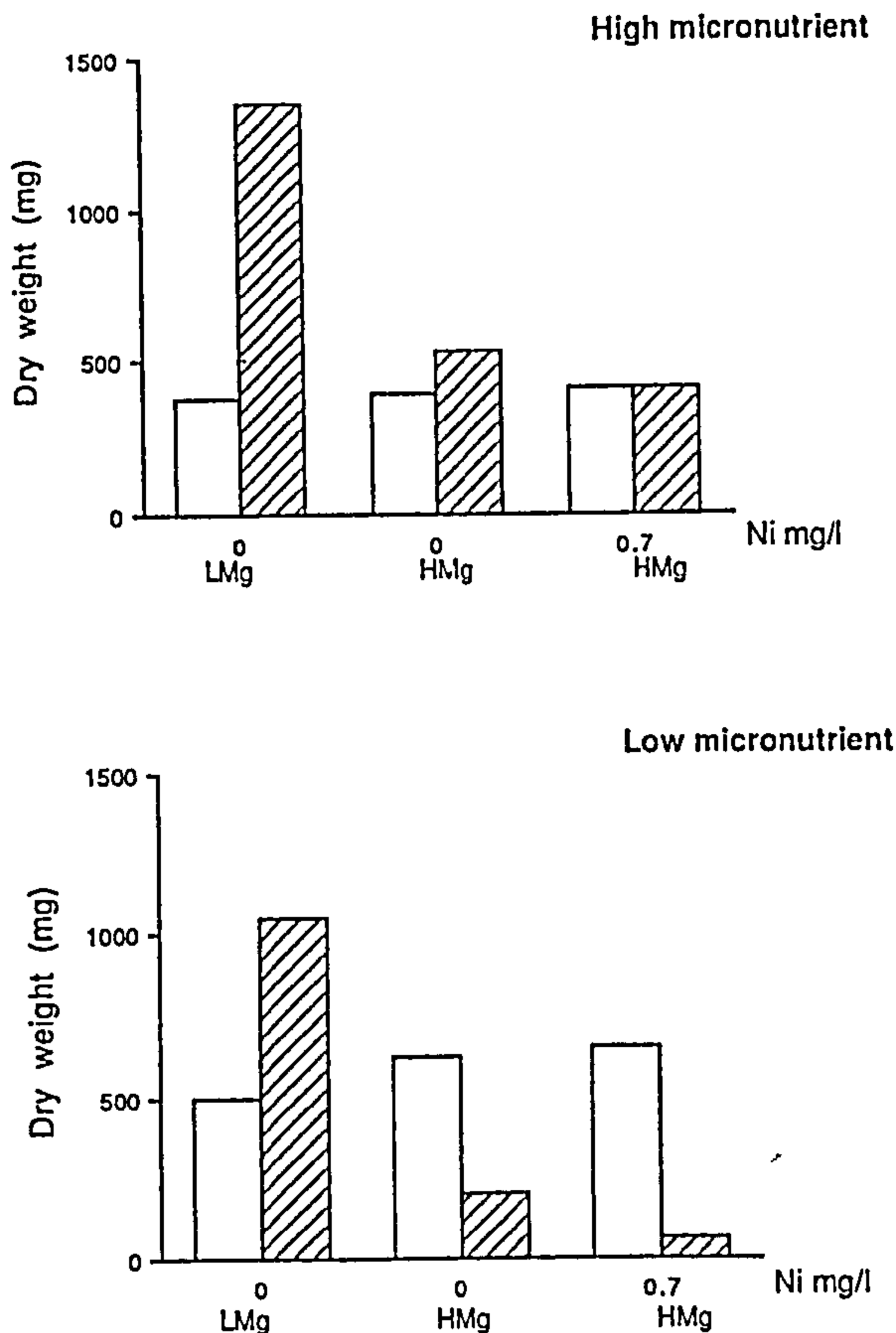


Figure 3 The growth of *Festuca rubra* in simulated Meikle Kilrannoch, Scotland, soil solutions at: two magnesium concentrations, low (LMg) and high (HMg); with ($0.7 \text{ mg l}^{-1} \text{ Ni}^{2+}$) and without added nickel; and at low and high micronutrient concentrations. The HMg, $0.7 \text{ mg l}^{-1} \text{ Ni}^{2+}$, low micronutrient treatment simulates the soil solutions most closely (from Johnston and Proctor²¹).

Various mechanisms of internal cycling of elements in short supply exist and may be well-developed on ultramafics. Vergnano Gambi *et al.* (this volume) have drawn attention to the possible role of root-surface phosphatases in the autolysis of root tissues.

Physical factors

Water shortage is a key factor in causing a distinct ultramafic vegetation and has certainly been viewed in this light since at least as early as Pančić⁹³. Armstrong and Huenneke (this volume) have given an account of the changes resulting from a four-year drought on Jasper Ridge, California. Perennial grasses showed few changes whereas the occurrence of annual grasses changed dramatically and their success in dry conditions depended on seed size. The invasion of the ultramafic area

by alien grasses was reduced by drought: Armstrong and Huenneke comment "the most common native annual grass, *Vulpia microstachys* tolerated the combination of drought and serpentine substratum much better than the non-native annual grasses". On Mount Bloomfield on Palawan in the Philippines drought seems to be mainly responsible for the small stature of the vegetation (A. J. M. Baker, L. A. Bruijnzeel and J. Proctor, in preparation) (Fig. 1).

Interactions between nutrients and drought have been described by Turitzin⁹⁴ in California. In Pennsylvanian ultramafic barren areas, growth of indigenous ultramafic species is favoured when moisture and nutrient supply are low but within a particular range of moisture and nutrient availability weedy species can co-exist with them⁹⁵.

In Shetland, within 2 km of Britain's wettest meteorological station, drought interacting with mineral nutrient shortage seems to be the cause of the barrenness on the ultramafic Keen of Hamar^{12,32}. There must be considerable scope for water and nutrient addition experiments of the type employed by Grime and Curtis⁹⁶ in their studies of grasslands in Derbyshire in England. They showed that additional water or additional phosphorus could improve growth and survival of the grass *Arrhenatherum elatius* in shallow limestone soils. They claimed that phosphorus addition stimulated root growth which enabled plants to get more water.

In Zimbabwe the dryness of the soils on much of the Great Dyke is a likely cause of its treelessness. Proctor and Craig⁹⁷ found well developed riverine forest on soils that were as high in exchangeable nickel and had as high Mg/Ca quotients as those in the surrounding grasslands on the Great Dyke. Robertson (this volume) has proposed an alternative explanation for the Zimbabwean situations which involve sequential effects of nickel toxicity and drought.

The xeromorphy of many ultramafic plants finds a ready explanation if we emphasize the importance of drought in their environment. Furthermore, any feature which leads to a reduction of transpiration will reduce the mass-flow delivery of soil solution toxins to the root surface. Kinzel⁴⁹ ascribed the relatively low magnesium concentrations in some Swiss ultramafic needle-leaved conifers to this effect.

We must not over-generalize about drought and it must be said that there are soligenous mires on some Scottish ultramafics⁹⁸ which provide the only floristic indication of the underlying geology. Furthermore, Kruckeberg (this volume) and Callizo (this volume) have shown that many of the ultramafic endemics in California are associated with soligenous mires (seeps). The hydrology of ultramafic areas needs much further work.

At high altitude the effects of frost heaving are important. Rune¹³ pointed out that shallow ultramafic soils are particularly prone to this type of activity. It can certainly uproot plants and also by its churning action will continually rejuvenate soils. It seems possible that the extremely magnesium toxic site at Meikle Kilrannoch in Scotland owes some of its effect to cryoturbation²⁰.

Evolution

We know most about evolution on ultramafics in California where Kruckeberg has given important lucid accounts (most recently 1992³). Progress towards an

understanding of evolution on ultramafics can only take place slowly since the case of each species must be investigated separately. We accept the viewpoint of Stebbins⁹⁹: "Like every other problem of evolution, that of the nature and occurrence of rare species is not a simple one that can be solved by applying indiscriminately one or few general principles". It is worth stressing at this point that all aspects of the study of ultramafics are dependent on taxonomists and the need for continuing taxonomic work (Edmondson, this volume) cannot be too strongly emphasized.

There are many formidable problems which presently defy explanation. We shall just outline one of these, the problem of endemism on tropical ultramafics. It is difficult to understand why some areas are rich and others poor in endemic species. In New Caledonia, ultramafic endemism is uniquely well developed. Out of 944 species of ultramafic maquis vegetation 724 (77%) are restricted to ultramafics. For forest there are 1511 species (including 77 epiphytes) of which 415 (27%) are restricted to ultramafics. Cuba is also rich in ultramafic endemics and Borhidi⁴ has given an account of the 920 endemics (one-third of the endemic flora) on ultramafics (7% of the land area) in that country (curiously many of the Cuban ultramafic species are thorny whilst this feature is restricted to only one species of *Capparis* and branched succulents in New Caledonia). Borhidi showed that the age of the ultramafic region is very important in accounting for the endemics. Of 24 endemic phanerogamous genera, twenty-two are restricted to surfaces which are several million years old. These surfaces have 86% of the 920 endemic species. The younger surfaces (< 1 m years old) have no endemic genera restricted to them and only 14% of the endemic species, even though they account for 36% of the total ultramafic area. Elsewhere in the tropics the endemism picture is different.

Proctor *et al.*¹⁵ reported only two large tree species restricted to ultramafics in Sabah, an area where tree speciation is among the highest in the world. Jaffré⁷ commented that in New Caledonia a high proportion of the large tree species are not restricted to ultramafic soil. Why this life form should show reduced speciation is not clear (there are many tree endemics in Cuba⁴) but it may be part of the explanation for the paucity of endemics in the study of Proctor *et al.*¹⁵. Over much of the rest of the tropics, ultramafic endemics of all life forms seem relatively rare. A. D. Bradshaw (personal communication) has suggested that this may sometimes result from past mass extinctions in the wake of great climatic changes since escape by migration might be impossible for plants highly adapted to restricted and ultramafic habitats. Batianoff *et al.*³⁰ reported 373 vascular plant species on ultramafics in open woodland savanna in eastern Australia. Of these only 13 (3.5%) are endemic. In Zimbabwe, of 322 species listed for the Great Dyke ultramafics, only 20 (6.2%) species, or plants of putatively lower formal taxonomic rank, were endemic¹⁰⁰. Wild and Bradshaw¹⁰¹ have shown that these are mainly likely to be ancient endemics since for several the nearest relatives are far away. In the original list of twenty¹⁰⁰, are forms of two species *Helichrysum pachyrhizum* and *Merremia pes-draconis* which Wild and Bradshaw¹⁰¹ included in a list of six species which showed some degree of morphological and probably genotypic differentiation in ultramafic populations. These two have now been given specific status as *Helichrysum serpentinicola* and *Merremia xanthophylla* and are perhaps the best candidates for neo-endemic species status in Zimbabwe. Most Zimbabwean species of the ultramafics are *bodenvag* and are often not spatially isolated from their

populations on non-ultramafic soils. There is no evidence of morphological divergence or the evolution of genetic isolating mechanisms in these species (G.C. Craig, personal communication). However, nickel-tolerant races have been demonstrated in some of the few Zimbabwean species investigated in this respect¹⁰²⁻¹⁰⁵. G.C. Craig (personal communication) believes such nickel tolerance may be more widespread on Zimbabwean ultramafics although so far the few examples from that country remain the only tropical examples of infraspecific differentiation in nickel tolerance. It seems as though the ultramafics of the Great Dyke are largely colonized by two extremes: ancient palaeoendemics and undifferentiated (or rarely morphologically differentiated) races (whose adaptations need clarification) of widespread species. As Wild and Bradshaw¹⁰¹ have commented: the shortage of neo-endemic species is "not easy to reconcile with present ideas on the ease with which parapatric speciation is supposed to be able to occur in adjacent but dissimilar habitats".

Among the promising recent papers which have made some contribution to the understanding of other problems of evolution on ultramafics are: the breeding experiments of Main¹⁰⁶ who has shown that magnesium tolerance has a genetic basis; the breeding experiments of Macnair (this volume) on *Mimulus* species; the electrophoretic studies of enzymes by Furnier and Adams¹⁰⁷ on *Pinus jeffreyi*; similar techniques and DNA restriction site analysis used by Westerbergh on *Silene dioica* (this volume) and Jain on *Helianthus* (this volume); the experimental investigations of ultramafic resistance in mosses, pioneered by Shaw¹⁰⁸; the detailed distributional study of nickel accumulators (Reeves, this volume); the investigations of Reeves and Baker⁶² on showing constitutional ultramafic tolerance with a non-specific metal detoxification system in both ultramafic and non-ultramafic races of *Thlaspi goesingense* in Austria (although their results contrast with many e.g. Goodwin-Bailey *et al.* (this volume) which failed to show constitutional tolerance in *Armeria maritima*); and the recognition of the role of nunataks in the ultramafic flora of Newfoundland (Ref. 9 and this volume).

Concluding remarks

Ultramafics are now emerging as a neglected area of this planet and large numbers of them are undescribed biologically. Their importance far outweighs the less than 1% of the earth's land surface which they occupy. We have tried to point out some ways forward for a better understanding of them, bearing in mind that generalizations are difficult because of the complex and interacting variables involved. We have mentioned many of these in this paper but have largely neglected biotic influences which can be overriding³³. Mycorrhizal studies on ultramafics have been few although there are strong indications¹⁰⁹ that ultramafic plants may be highly mycorrhizal. The experimental studies made so far^{110,111} have shown the potential for mycorrhizal involvement rather than quantified its effect. The demonstration by Bradley *et al.*¹¹² that mycorrhizal fungi may be involved in the avoidance of copper and zinc toxicity in *Calluna vulgaris* (Ericaceae) and related species suggests a possible detoxifying role for microorganisms in ultramafics and should be further investigated.

Nothing was said at the Conference on the role of animals on ultramafics and animal data are sparse. The important effects of gophers on the Jasper Ridge site in California are now well documented¹¹³⁻¹¹⁶. Other animals may be involved on Jasper Ridge. J. Proctor (unpublished) showed that seeds of a common alien grass species (*Avena fatua*) were removed from the ultramafic soil surface by unknown animals before they germinated. Populations of litter invertebrates have been investigated by Leakey and Proctor¹¹⁷ for Gunung Silam, Sabah and their preliminary studies should be extended.

There is no doubt that ultramafic areas pose stress to plants. However while a large body of plant physiological data now exists for plants growing in other stressful environments with abiotic controlling factors¹¹⁸, barely any other aspect than that of mineral nutrition (in a broad sense) has been researched for plants growing on ultramafics. The number of works on the photosynthetic ecology of ultramafics is especially few¹¹⁹⁻¹²¹. As Vergnano Gambi⁵ has suggested, future work should ascertain the occurrence of C₄ photosynthesis on ultramafics, and we would add, the relationship of the mineral nutrition of the ultramafic plants with their photosynthetic ability. At the interface between genetics, biochemistry, and ecology, ultramafics provide ideal materials for study. As Kruckeberg³ has recently eloquently expressed "So clear is the distinction between ultramafic and non-ultramafic existence, that its study at the molecular level is bound to yield understanding of the gene-to-environment system. Why plants grow where they grow, attacked at the molecular level, is ripe for investigation".

In many countries ultramafic rocks are of sufficient extent to be of major economic importance, in others they are neglected wastelands, in still others they bear valuable forests. In all cases they are going to present formidable problems of conservation. Apart from being reservoirs of rarities they have untapped potential to contribute to fundamental research. The conservation of ultramafic areas is an issue which should have high priority and Jain (this volume) has given a useful example to strengthen conservation arguments when he showed that the genes from a Californian endemic *Helianthus* species were being bred into commercial sunflowers. The problems of understanding plant evolution on ultramafics are difficult but clearly defined and their solution will contribute to our understanding of evolution in general. Our hope is that the studies we have discussed will continue to flourish and that this Conference will be the first of many on ultramafic rocks and their vegetation.

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