A multivariate approach to plant mineral nutrition: dose—response relationships and nutrient dominance in factorial experiments

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Canonical variates analysis was used to compare the effects of fertilization on the concentrations of five mineral elements (N, P, K, Ca, and Mg) in young shoot tissues of six tundra plant species of three different growth forms. There were two specific objectives: (i) to determine whether it was possible to describe meaningful dose—response relationships in a multivariate response to fertilization, and (ii) to determine the multivariate effect of N plus P fertilization in comparison with the effects of N or P added alone. The results showed that low levels of N—P—K fertilization caused a shift in multivariate nutrient content that was intermediate between the control values and the shift caused by high fertilization, and in the same direction as the latter. In a June harvest, the effect of N plus P fertilization was very similar to the effect of N fertilization alone. However, in August the N plus P effect was dominated by the response to P alone. In all of the analyses, the fundamental similarities and differences among unfertilized plants of each species and growth form were maintained under fertilization.

Shaver, G. R., et M. J. Lechowicz. 1985. Un approche multivariée pour comparer les effets de la fertilisation sur la concentration de cinq éléments minéraux (N, P, K, Ca et Mg) dans les tissus des jeunes pousses de cinq espèces végétales de la toundra, représentant trois formes de croissance différentes. Les deux objectifs spécifiques étaient les suivants: (i) déterminer s’il est possible de dégager des relations dose—réponse intelligibles dans une réponse multivariée à la fertilisation et (ii) déterminer l’effet multivarié d’une fertilisation azotée et phosphorée, comparativement aux effets de l’apport de N seul ou de P seul. Les résultats montrent que de faibles niveaux de fertilisation par N—P—K provoquent un changement de la teneur multivariée en éléments minéraux et que ce changement est moins important, mais dans la même direction que celui provoqué par des niveaux élevés de fertilisation. Dans une récolte effectuée en juin, l’effet d’une fertilisation par N et P est très semblable à celui d’une fertilisation par N seulement. Cependant, en août, l’effet de N et P est dominé par la réponse au phosphate seul. Dans toutes les analyses, la fertilisation ne change pas les similitudes et différences fondamentales qui existent entre les plantes non fertilisées des différentes espèces et formes de croissance.

Introduction

Plant mineral nutrition has a long history of research, and many of the basic mechanisms of mineral uptake, metabolism, and allocation are well-known. However, at present few generalities are possible about how two or more nutrients interact in controlling plant growth (Salisbury 1975). Few predictions are possible about the overall patterns of plant mineral nutrition in diverse habitats or plant growth forms (Chapin 1980). Over the past decade, considerable progress has been made by application of multivariate statistical methods, particularly canonical variates analysis (sensu Gittins 1979), to the problems of nutrient interaction and overall plant nutrient status (Woodwell et al. 1975; Garten et al. 1977; Garten 1978; Marrs 1978; Gittins 1979). For example, in Alaskan tundra the major plant growth forms are distinctive from each other in terms of their overall patterns of mineral nutrient accumulation (Lechowicz and Shaver 1982). Deciduous, evergreen, and graminoid species can be distinguished by growth form in a multivariate comparison of N, P, K, Ca, and Mg concentrations. The differences among growth forms in these Alaskan plants are maintained both through the growing season and in response to fertilization. The differences in mineral nutrition are important because they help to explain variation in the relative abundances of growth forms along gradients of nutrient availability and utilization patterns (Stoner et al. 1978; Webber 1978; Miller 1982). Presumably, differences among growth forms in overall mineral nutrient content reflect differences in nutrient requirements (Woodwell et al. 1975; Garten 1978; Marrs 1978).

The grouping of species by growth form in terms of their mineral nutrition is apparent only with a multivariate approach such as canonical analysis (Lechowicz and Shaver 1982) or principal component ordination (Woodwell et al. 1975). Univariate analyses of the same data show numerous similarities and differences among graminoid, deciduous, and evergreen species, but do not allow objective distinction among growth forms (Shaver and Chapin 1980). This paper further extends the use of the multivariate approach to interpreting the responses of Alaskan tundra plants to fertilization (Shaver and Chapin 1980; Lechowicz and Shaver 1982).

The long-term goal of this research is to determine the underlying patterns of plant mineral nutrition that might explain differences in the distribution and abundance of the graminoid, evergreen, and deciduous growth forms in tundra regions (Shaver and Chapin 1980; Chapin and Shaver 1985). In this paper we use a series of canonical variates analyses to answer two principal questions. First, does a low level of N—P—K fertilization cause a change in the overall plant nutrient content that is less than the change caused by a higher level of N—P—K fertilization? Unnumerous studies have shown that this is usually (but not always) true for single elements, but such dose—response relationships have rarely been discussed in the multivariate sense. Second, how does the effect of N plus P
fertilization on overall plant nutrient content compare with that of N or P added alone? The additive or interactive effects of individual element availability an overall nutrient content are also not well understood.

Methods

Fertilization experiment

The site of the fertilization experiment was Eagle Creek, Alaska (65°10'N, 145°30'W, elevation 760 m). The general ecology of the site and its surroundings are described in detail in a series of papers edited by Miller (1982). The vegetation is typical cottongrass-tussock tundra dominated by Eriophorum vaginatum L. ssp. spissum (Fern.) Hult. (Shaver and Cutler 1979). The main experiment was a complete factorial N−P−K fertilization experiment with five replicate 2 × 2 m plots randomly selected for each treatment (Shaver and Chapin 1980). Nitrogen was applied as NH₄NO₃ at 25 g m⁻², phosphorus as superphosphate at 25 g m⁻², and potassium as K₂O at 31.6 g m⁻². The rates of application were chosen for comparison with earlier studies in Sweden and Great Britain (Tamm 1954; Goodeman and Perkins 1966a, 1966b). The fertilizers were applied once on 8 July 1976.

Two other treatments were also started on 8 July 1976. These were a low-level N−P−K fertilization (one-fifth of the amount applied in the high N−P−K fertilization) and a sugar application of 100 gm⁻². The purpose of the sugar application was to stimulate microbial demand for soil nutrients by providing microbes with a ready carbon source, thus increasing nutrient availability to plants (Turner and Olsen 1976; Turner 1977). In a previous paper (Shaver and Chapin 1980), we showed that the sugar treatment caused a significant reduction in tissue concentrations of N and P in some species. However, not all species or elements showed reduced concentrations in the univariate analyses.

Tissues of five species were harvested on 14−15 June and on 17 August 1977: the evergreens Vaccinium vitis-idaea and Ledum palustre, the deciduous shrubs Vaccinium uliginosum and Betula nana, and the graminoid E. vaginatum. A second graminoid, Carex bigelowii, was included in the August harvest. The harvested material included the most recent year’s production of leaf plus associated stem tissues (Shaver and Chapin 1980). The tissues were dried and analyzed for concentration of N and P by standard Technicon autoanalyzer methods and of K, Ca, and Mg by atomic absorption spectroscopy.

Canonical variates analysis

The data were analyzed by canonical variates analysis (sensu Gittins 1979) using the routine in the Statistical Package for the Social Sciences, Release 9.1 (Nie et al. 1975). In canonical variates analysis, the multivariate observations (grouped in diverse ways, as described below, to elucidate different aspects of nutrient response) are located along canonical axes to minimize the variance among observations within a group but to maximize variance between groups. This is achieved by determining a unique set of canonical coefficients for each character in the multivariate observations; these coefficients weight the actual character values for each observation to give greater emphasis to characters better able to separate the defined groupings of observations from one another. The relative values of these canonical coefficients can be used to help interpret which characters contribute most to placing observations along each canonical axis. Toward this end the coefficients relativized to the maximum absolute value on each canonical axis appear in the figures (Lechowicz and Shaver 1982). Additional explanation and mathematical detail of the use of canonical variates analysis in ecology may be found in Kowal et al. (1976), Gittins (1979), and Williams (1983).

Three different sets of analyses were completed, each using different subsets of the total data set to answer particular questions. All of the analyses used the untransformed tissue concentrations of five mineral elements (N, P, K, Ca, and Mg) to discriminate among replicate samples grouped by species, treatment, and harvest date. In the first analysis, only the data from the control plants were used. This analysis provided a simultaneous comparison of growth forms at each harvest, and illustrated background changes in the nutritional relationships within and between growth forms from harvest to harvest. The second set of analyses was divided into two parts, one using only data from the June harvest and the other only data from the August harvest. Both parts used the data from the controls and the low N−P−K, high N−P−K, and sugar treatments for each species. These analyses were used to determine multivariate dose−response relationships to N−P−K availability. The third set of analyses also was separated into two parts, one for each seasonal harvest. In each season, tissue nutrient profiles in the unfertilized controls for each species were compared with those in the corresponding N, P, and N plus P fertilized samples. These two analyses illustrated the multivariate response of the species in each season to changing proportions of available N and P.

Results

Comparison of unfertilized plants

In the analysis of control samples (Fig. 1), the species and growth forms were arranged along the first canonical axis (69.9% of total variance) roughly in order of increasing Ca concentration and decreasing N concentration (compare relativized coefficients and see Tables 1 and 2). Carex bigelowii also was distinguished by its high K concentrations. The second canonical axis (17.1% of total variance) separated the deciduous species from the other two growth forms and increased discrimination of June from August samples, mainly on the basis of P concentration. All five species harvested at both dates moved down the second canonical axis from June to August, primarily because of decreases in P concentration.

Changes in overall mineral nutrition from June to August are shown in Fig. 1 by connecting the group centroid for each species in June to its centroid in August, using an arrow pointing from June to August. The arrows suggest that overall nutritional differences among species were reduced in August, mainly because of a changing balance of Ca to N and convergence of P concentrations. In August compared with June,
evergreens had a lower Ca:N ratio, while the deciduous species had a higher Ca:N ratio; the Ca:N ratio in *E. vaginatum* changed little.

**Levels of fertilization**

Species relationships in comparison of the effects of levels of fertilization (Fig. 2) were similar to the comparison of controls alone (Fig. 1), suggesting that fundamental differences in mineral nutrition among species and growth forms were maintained despite changes in nutrient availability. The first two canonical axes explained 88.5% of the total variance in June (Fig. 2A) and 78.4% in August (Fig. 2B). While the differences among species and growth forms were most apparent along canonical axis I, treatment effects were most apparent along canonical axis II (Figs. 2A, 2B). The second canonical axis explained only about one-third as much of the variance as the first.

In both June and August, Ca concentration was again the most important element in discrimination along the first axis (Figs. 2A, 2B; Tables 1, 2). However, the relative weightings of N and P along both axes were very different at the two sample dates. In June, N concentration was negatively weighted on the first canonical axis and was strongly positively weighted along the second axis. The opposite was true for N weightings in August, and the relative importance of N was less. P concentration, on the other hand, was the dominant element along axis II in August, and also was strongly positively weighted along axis I then.

The multivariate nutrient content of plants under the low N–P–K fertilization was intermediate between the control and high N–P–K treatments (Figs. 2A, 2B). The only major exception was *E. vaginatum* in June, for which the low N–P–K treatment had little if any effect (Fig. 2A). In most cases the group centroid for the low N–P–K treatment was located almost directly along a line between the centroids for the control and the high N–P–K treatments; this indicates a simple
FIG. 2. Canonical analysis of changes in nutrient concentrations of young shoot tissues after treatment with differing levels of nutrient availability. Responses for species representing three growth forms, symbolized as in Fig. 1, are shown for June (A) and for August (B) of the year following treatments. For each species, the arrows connect the group centroids in the following order: (1) a sugar treatment intended to reduce available nutrients, (2) an untreated control (open symbol), (3) a low-level N–P–K fertilization, and (4) a high-level N–P–K fertilization. The relativized coefficients indicate the contribution of each measured tissue nutrient to placement of the species along the two canonical variate axes; see Lechowicz and Shaver (1982) for explanation of canonical analysis and the text for discussion. In both sections of the figure, both canonical axes are highly significant by the Wilk’s λ criterion (p < 0.0001).

quantitative, although not always linear, response to increasing nutrient availability.

Effects of the sugar treatment were unclear. In some cases, such as for L. palustris in June (Fig. 2A) and C. bigelowii in August (Fig. 2B), the sugar treatment affected overall nutrient status in a way that was nearly opposite to the N–P–K fertilizations. These responses were consistent with our original expectations. However, in most cases the sugar treatment effects were either nonsignificant or inconsistent both with the fertilizations and among species.

Additive or interactive fertilization effects

In the comparison of N, P, and N–P fertilization effects, species relationships were again similar to those in the comparison of the controls (Figs. 1, 3). The first two canonical axes explained 94.4% of the variance in June and 82.0% in August (Figs. 3A, 3B). The species differences were again greatest along the first axis, but in contrast to the effects of N–P–K fertilization (Fig. 2), single-nutrient treatments caused major shifts in group centroids along both canonical axes (Fig. 3).

In June, the N fertilization treatment and the N concentration response were dominant (Fig. 3A; Table 1). The group centroids for the N treatment shifted from the controls in the same direction for all species, while the P response was smaller and less consistent. Most importantly, the centroids for the N–P fertilization treatment shifted a distance and in a direction
almost identical with the shift in the N treatment, suggesting that in combination with N, P had little or no effect in June. N concentration in the tissues was the most important weighting factor along axis II and was second only to Ca along axis I.

In August, the relative dominance of N versus P was reversed (Fig. 3B, Table 2). The P treatments in August caused shifts in the group centroids that were much larger and more consistent among species than the N treatments. Group centroids for the N−P treatments were much closer to the centroids for the P treatments, although the similarity in response of the P and N−P treatments in August was not as great as for the N and N−P treatments in June. P concentration in August was relatively most heavily weighted along axis II but followed Ca and Mg in importance along axis I.

Discussion

The comparison of effects of different levels of fertilization showed that intermediate states of plant nutrition exist in the multivariate as well as the univariate sense. In most cases the group centroid for the low N−P−K fertilization was located directly between the control and the high N−P−K treatments. The sugar treatment, which was intended to reduce nutrient status of plants by increasing soil microbial demands, had uncertain effects.

The most likely cause of uncertainty in the sugar response was the design and sampling schedule of our experiment. The plants were not harvested until almost two full growing seasons after sugar application, which would allow time for a rapid nutrient immobilization as a result of stimulation of microbial growth, followed by a crash in microbial populations and perhaps even a net increase in nutrient availability by the time of the harvest. However, we did notice a significant decrease in the concentrations of some elements in some species in the univariate analyses (Shaver and Chapin 1980), and Marion et al. (1982) were able to detect a depression of 15N uptake in the same species at Eagle Creek after an analogous treatment with cornstarch. A key question for future research is the timing of plant uptake processes relative to microbial immobilization and release. We know that in tundra plants nutrient uptake processes and utilization in growth may not even occur in the same growing season (Shaver et al. 1986), and thus the occasional negative effects of the sugar treatment on concentrations of elements were not surprising. The lack of a consistent response to sugar does not diminish the clear and consistent dose−response relationships we observed for fertilization.

The comparison of effects of the N, P, and N−P treatments showed that one of the added nutrients (N or P) was always dominant over the other. When both N and P were added to the field plots, either the nutrient status of the plants showed no effect of the added subdominant element or the result was roughly the vector sum of the responses to the individual nutrient additions. The first type of response was more typical in June, and would suggest a kind of multivariate interaction in the response of plant nutrient status to changes in nutrient availability. The second type of response was more common in August, and suggests that the effects are additive. This type of analysis must be performed on other data sets before any consistent patterns emerge relating to the interaction or additiveness of single fertilizer effects.

It is clear, though, that N fertilization consistently caused the greatest multivariate responses in the June harvest, while P fertilization was dominant in August. In the tissues, the most responsive elements were N in June and P in August. All fertilizers had been applied for 1 full year before the June harvest so it is unlikely that differences in mobility of fertilizers could have caused the seasonal differences in responsiveness. The cause of the shift from N to P dominance is not clear, but it is most likely related to seasonal changes in N versus P uptake, and allocation to the young, growing tissues sampled here. These seasonal changes in fertilization responses were paralleled by seasonal changes in the relative importance of N versus P in the canonical variates analysis of control plant tissues, and in earlier univariate analyses (Shaver and Chapin 1980).

The fertilization treatments had only a small effect on the multivariate similarities and differences between species. Rather, the underlying pattern of relationships among unfertilized control plants was maintained under fertilization. This suggests that despite major changes in the ratio of availability of N, P, and K, each species shifted its ratio of accumulation of these elements (plus Ca and Mg) to a much lesser degree. Shaver and Melillo (1984) obtained similar results for N:P ratios in a phytotron experiment. The implications of this result are that nutrient relationships in uptake by a whole vegetation will be strongly affected by the species composition of that vegetation, irrespective of the relative availabilities of different elements.

The robustness of the multivariate species and growth form relationships also reflects our earlier observation that the growth of all species was about equally limited by N and by N plus P at Eagle Creek (Shaver and Chapin 1980). It seems reasonable to expect that if all species are initially limited by the same combination of nutrients, then fertilization should cause all species to move in the same trajectory across the plane represented by the first two canonical axes (e.g., Fig. 2), thus maintaining roughly the same initial species relationships. On the other hand, if some species were N-limited and others were P-limited, then one might expect a different trajectory for each group.

Initially we predicted that (i) each of the dominant species in the community would be limited by a different combination of N, P, and K, and (ii) the decidious species would be the most responsive of the three growth forms to fertilization (Shaver and Chapin 1980; Lechowicz and Shaver 1982). These predictions were based on the expectation of some niche diversification in nutrient use by these plants, and on previous research which had established a higher nutrient requirement for rapidly growing deciduous species (Chapin 1980; Chapin et al. 1980; Chapin and Bieleski 1982). Instead, we found no difference in the pattern or degree of nutrient limitation at Eagle Creek (Shaver and Chapin 1980), a result which is supported by our multivariate analyses of plant nutrition. In more recent work at Toolik Lake, Alaska, we have found that light and temperature regime must also be considered, in interaction with nutrient availability, to explain the relative abundance and limits to production of the various species (Chapin and Shaver 1985). In the future we believe that multivariate analyses of nutrient content plus the major mobile carbon pools, and their interactions, should help us finally to understand the underlying nutritional mechanisms responsible for controlling abundances of species and growth forms in Alaskan tundra.

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