*Acta Bot. Neerl.* 43(4), December 1994, p. 383–392

# Effects of nitrogen, phosphorus and potassium fertilization on field performance of *Dactylorhiza majalis*

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## SUMMARY

The effects of nitrogen, potassium and phosphorus fertilization on performance of *Dactylorhiza majalis* (Rchb.f.) Hunt & Summerh. were studied in a *Juncus acutiflorus*-dominated hayfield. Frequency, total shoot biomass and flowering incidence decreased as a result of both nitrogen and phosphorus application. Nutrient additions also affected yields of species showing positive and negative association with orchid performance. These changes were sufficient in explaining the nitrogen and phosphate response of orchid shoot biomass and extent of flowering. The most important competitors in this respect were *Rumex acetosa* and *Holcus lanatus*. The negative overall N- and P-effect on orchid frequency seemed to be independent of changes in the surrounding vegetation however. The relative importance of nutrient limitations and additions is discussed in relation to the species decline in frequency and to its reappearance in grasslands under restoration management with decreasing nutrient availability.

*Key-words:* nitrogen, phosphate, potassium, *Dactylorhiza majalis* (Rchb.) Hunt & Summerh., restoration ecology.

## INTRODUCTION

In general, European terrestrial orchid species are declining rapidly in frequency of occurrence. After 1950, *Dactylorhiza majalis* s.l.* was recorded in 408 5 × 5 km sequences of the 579 from which it was known in The Netherlands before then (Mennema *et al.* 1985). Probably only the minority of these cases concerns the subject of this study, *D. majalis* (Rchb.) Hunt & Summerh, s.s.† For those species which occur in marshy hayfields, lowering of groundwater tables and application of fertilizers have been considered major causes of decline in The Netherlands (Mennema *et al.* 1985). Both types of agricultural interference have pronounced effects on nutrient availability,

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*Including *D. praetermissa* (Druce) Soó.

†*D. majalis* s.s. is reported from 154 5 × 5 km squares in the period 1980–94 (C.A.J. Kreutz, unpublished data).

the first one especially on nitrogen availability via enhanced mineralization rates. Accumulation of nitrate in the top layer of the soil has been demonstrated in drained parts of *Cirsio-Molinietum* stands (Grootjans *et al.* 1986) in the immediate vicinity of populations of four species of marsh orchids including *D. majalis*. Similar effects were observed in *Calthion palustris*‡ communities (Grootjans *et al.* 1985) of which *Dactylorhiza majalis* is a character species and in which several other marsh orchids regularly occur.

Increase in nutrient supply can affect growth and occurrence of a given species within plant communities in several ways. When quantities are limiting, growth can be enhanced while in other situations supra-optimal or even toxic levels may inhibit it. These effects are dependent on the physiological characteristics of the particular plant species.

Nutrient effects may also be expressed in an indirect way, by means of shifting the competitive balance between the target species and the other plant species by which it is accompanied. For example, neighbouring species showing a considerable growth response to nutrient addition, might outcompete the target species for light.

In the present study the response of the broad-leaved marsh orchid (*Dactylorhiza majalis* (Rchb.) Hunt & Summerh. ss.§) to artificial fertilization with the major macronutrients is investigated in the field. Its aim is to verify whether increased nutrient availability could indeed be a cause of the species' decline. Furthermore, an attempt is made to assess the extent to which overall nutrient effects are direct, physiological effects affecting growth of the individuals, or indirect ones affecting the competitive balance with other species. Results of this field study have been used for design and interpretation of laboratory experiments with single orchid species, in which interaction with other plant species is not included.

## MATERIALS AND METHODS

### *Species characteristics*

*Dactylorhiza majalis* is a perennial with a prolonged heterotrophic juvenile phase. The species hibernates as a dormant, finger-like divided tuber. The shoot emerges in early spring while, simultaneously, an already present tuber initial for the next season enlarges. Peak above-ground biomass is reached at the end of the growing season when mature individuals flower, usually from late May to the first weeks of June. In related species such as *Orchis morio, O. mascula* and *Platanthera bifolia*, reallocation of reserves from the old to the new tuber has been demonstrated at the end of the growing season (cf. Ernst & Rodriguez 1984). This means that changes in edaphic factors or responses to accompanying changes in vegetation composition will rather be expressed in next year's plant survival or biomass, and that the current year's performance is largely determined by reserves present in the old tuber. Therefore, the experimental period should at least span two growing seasons, of which one is needed solely for an optimal expression of effects. In this study 2 years of fertilizer application preceeded estimation of the effects on performance of *D. majalis* and correlation with vegetation parameters was based on species yields of the preceeding growth season.

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‡Syntaxonomical nomenclature follows Westhoff & den Held (1975).

§Nomenclature of plant species follows Heukels & van der Meijden (1983) except for orchid species which are named according to Landwehr (1977); *Dactylorhiza majalis* and *D. praetermissa* are treated as separate species.

### *Study site*

The experimental area is situated at the Anloër Diepje (53°05′N, 6°40′E), which forms part of the brooklet system of the Drentsche Aa. The particular hayfield where the experiment was conducted was reclaimed from agricultural use in 1946, and has not been fertilized since. Hay-making has been continued; the sward is mown annually in the first weeks of August. The vegetation consisted of a *Juncus acutiflorus*-dominated grassland, and can be classified as a mature stage of a *Calthion palustris*-community. Densities of *D. majalis* were high, up to 12 individuals per $m^2$.

Both nitrogen and potassium were shown to be limiting for total standing crop of the vegetation on this site (Olff 1992). *In situ* nitrogen mineralization rates were very low (Olff *et al.* 1994). In a glasshouse experiment, joint limitation of nitrogen, phosphorus and potassium was demonstrated (Olff & Pegtel 1994).

### *Experimental design*

A part of the hayfield was divided into 1 $m^2$ sized plots, separated by 0·5 m. Each of these plots did or did not receive a fertilization of nitrogen (25 g N $m^{-2}$ $year^{-1}$ as $NH_4NO_3$), potassium (25 g K $m^{-2}$ $year^{-1}$ as KCl) and/or phosphorus (8 g P $m^{-2}$ $year^{-1}$ as $NaH_2PO_4$) in a full factorial design, resulting in eight different treatments. Treatments were assigned to five replicate plots at random. Nutrients were given as solutions (1 litre $m^{-2}$) in two subsequent doses that were supplied in the end of April and the beginning of May of both 1990 and 1991. Control treatments received demineralized water only.

Data on vegetation composition of the plots in 1991 were collected by analysing sods of 10 × 40 cm. Above-ground biomass of these samples was sorted to species. Dry weights of these fractions were determined after drying at 70°C. Other details on the experimental set-up and data concerning vegetation response to the nutrient additions are given by Olff (1992).

In June 1992, the effects of fertilizer application on performance of *Dactylorhiza majalis* were studied by counting the numbers of vegetative and flowering plants in the entire plots. Biomass of orchid shoots was determined after drying overnight at 70°C.

### *Statistical analyses*

*Selection of orchid performance measures and overall nutrient effects.* Six interrelated performance variables were determined: densities of vegetative and generative plants per $m^2$; total number of orchids per plot; average biomass of individuals within plots; total shoot biomass per plot; and the fraction of individuals flowering. Average biomass and flowering incidence were log-transformed to improve normality and homogeneity of variance required for subsequent analysis of variance. The other variables were square-root transformed.

To assess the extent of joint variation of these variables, principal component analysis (PCA) was applied on the correlation matrix. After VARIMAX rotation (see Hair *et al.* 1992), the most redundant variables were removed. The relation between plant size and probability of flowering was also tested more directly by means of logistic regression, using dry weights of the separate individuals as the independent variable. Overall nutrient effects on the remaining performance measures of *D. majalis* were established by subjecting the transformed variables to a multivariate analysis of variance (MANOVA). Comparisons between separate treatments were made by applying one-way analyses of variance at significance levels of 0·05.

*Orchid performance in relation to yields of co-occurring species.* With the aim to assess the extent to which nutrient effects in orchid performance could be explained from the response of the surrounding vegetation, the transformed performance measures were used as dependent variables in a canonical correspondence analysis (CCA). Standing crop and amount of litter in the preceeding season were included as covariates after log-transformation. Correlations between the variables were assessed using their canonical loadings on the main two axes. Following guidelines in Hair *et al.* (1992), absolute values of loadings exceeding 0·30 were considered significant. A similar procedure was used to detect correlations between orchid performance and log-transformed yields of separate co-occurring species. Nutrient effects on total standing crop, amount of litter and yields of relevant competitors were analysed by subjecting transformed yields to a MANOVA.

To correct nutrient effects on orchid performance for changes of relevant vegetation parameters, performance variables were adjusted for effects of log-transformed yields in a multivariate analysis of covariance (MANCOVA). Only those variables were incorporated as covariates that showed meaningful negative or positive associations to one of the orchid variables, as indicated by the CCA described above.

Differences between the outcome between MANOVA and MANCOVA can be attributed only to the collective effect of changes in biomass of all selected competitors. However, the importance of separate species can be estimated to some degree by comparing magnitude and direction of the nutrient effects to the ones observed for *D. majalis.*

## RESULTS

### *Selection of orchid performance measures and overall nutrient effects*

Densities of *D. majalis* were highest in the control plots receiving no nutrients, and in the plots that were fertilized with potassium only (Fig. 1, left-hand bars). Variation between plots was large, however, and differences between separate treatments were not significant. The fraction of flowering individuals within a plot was the highest in the control plots and differed significantly from plots treated with N and P. K-fertilization resulted in the highest total shoot biomass (Fig. 1, right-hand bars), which was significantly higher than the yields in all treatments in which N- and/or P were added.

Two dimensions of variation were found in the performance variables, covering, respectively, 60% and 19% of overall variance in the variables (Fig. 2). The largest amount of variance was found in orchid density, correlated to about the same extent to variation in both vegetative and generative plants. Effects on orchid frequency, therefore, cannot be ascribed to frequency changes of seedlings or adult plants, but seem to originate from almost identical responses of both categories. The fraction of flowering individuals was not correlated to the number of generative or vegetative plants, suggesting that changes in this variable cannot be attributed to effects on mature plants or seedlings. Flowering incidence, however, was related to the average biomass of individuals within the plot. Indeed, separate individuals were more likely to flower with increasing dry weight ($n=89$, $-2\mathrm{LL}^{*}=123{\cdot}4$, $P<0{\cdot}001$).

Total above-ground orchid biomass was correlated more strongly to average biomass of the individuals than to the number of individuals in the plots.

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[*] $-2\times$ log likelihood.

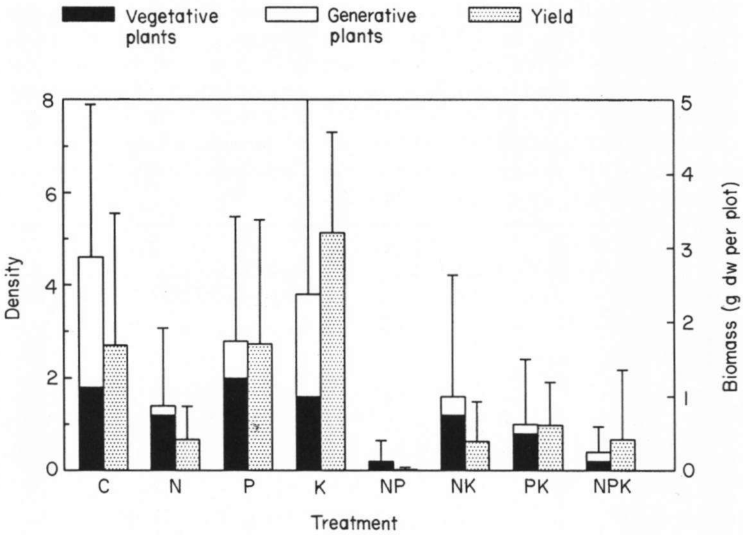

**Fig. 1.** Density (left-hand bars, left axis) and total above-ground biomass (g dry weight; right-hand bars, right axis) of *Dactylorhiza majalis* in control plots (C) and plots fertilized with nitrogen (N), phosphorus (P), potassium (K) or a combination of these. Means of five replicates are given, together with their standard devations. Numbers of orchids per plot have been separated in vegetative (■) and generative (□) plants; error bars indicate standard deviation of total number per plot.

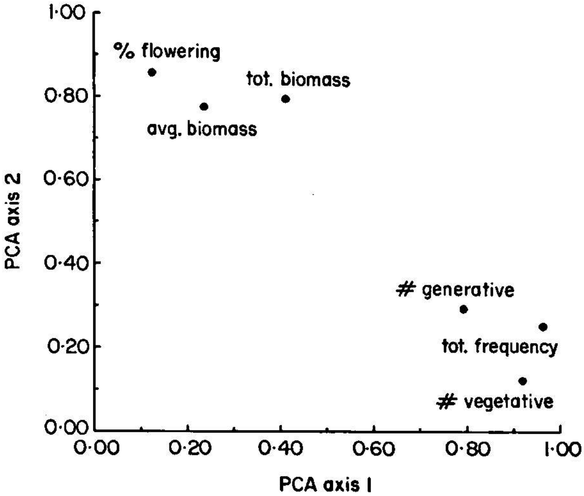

**Fig. 2.** Main dimensions of joint variation in variables measuring performance of *Dactylorhiza majalis*, as given by principal components analysis (PCA). Variables are abbreviated as follows: #vegetative, #generative=number of vegetative and generative plants, respectively, per plot (1 $m^2$); tot. frequency=total number of individuals per plot; tot. biomass=total above-ground biomass (g dry weight) of all individuals present in the plot; avg. biomass=average above-ground biomass (g dry weight) of individuals within a plot; % flowering=percentage of flowering individuals within the plots. Points represent loadings of the measured variables on the first two extracted axes.

Two highly redundant groups could be distinguished within the variables, one comprising of the density variables, and a second of average individual biomass and fraction of flowering individuals. Total frequency and fraction of flowering individuals

**Table 1.** Results of multiple analysis of variance (left) and covariance (right) of densities, total biomass and flowering incidence of *Dactylorhiza majalis* per test plot as dependent on the fertilization regime. All nutrient factors and interactions have been tested at one degree of freedom. Effects are given as estimates of change in the transformed variables. The MANOVA summarizes general nutrient effects on orchid performance; the MANCOVA gives nutrient effects corrected for changes of the most relevant co-occurring plant species

| | MANOVA | | | MANCOVA | | |
|---|---|---|---|---|---|---|
| Treatment | Effect | *F* | Sign. | Effect | *F* | Sign. |
| *Density* | | | | | | |
| N | −0·77 | 6·11 | * | −1·71 | 9·47 | ** |
| P | −0·64 | 4·19 | * | −1·17 | 5·74 | * |
| K | −0·30 | 0·92 | — | −0·57 | 1·95 | — |
| N × P | 0·22 | 0·12 | — | −0·10 | 0·02 | — |
| N × K | 0·69 | 1·22 | — | 0·03 | 0·00 | — |
| P × K | 0·14 | 0·05 | — | −0·57 | 0·60 | — |
| N × P × K | 0·35 | 0·08 | — | −1·18 | 0·61 | — |
| *Total biomass* | | | | | | |
| N | −0·48 | 18·55 | *** | −0·43 | 4·10 | (*) |
| P | −0·24 | 4·55 | * | −0·34 | 3·24 | (*) |
| K | 0·07 | 0·36 | — | 0·25 | 2·51 | — |
| N × P | 0·29 | 1·73 | — | 0·01 | 0·00 | — |
| N × K | −0·00 | 0·00 | — | −0·14 | 0·19 | — |
| P × K | −0·31 | 1·93 | — | −0·44 | 2·45 | — |
| N × P × K | 0·95 | 4·54 | * | 0·94 | 2·60 | — |
| *Flowering incidence* | | | | | | |
| N | −0·25 | 13·47 | ** | −0·18 | 1·94 | — |
| P | −0·19 | 7·53 | * | −0·14 | 1·50 | — |
| K | 0·02 | 0·09 | — | 0·12 | 1·55 | — |
| N × P | 0·19 | 1·92 | — | 0·19 | 1·32 | — |
| N × K | 0·21 | 2·28 | — | 0·41 | 4·67 | * |
| P × K | 0·03 | 0·06 | — | 0·08 | 0·24 | — |
| N × P × K | −0·12 | 0·19 | — | 0·13 | 0·15 | — |

*$0{\cdot}01 \leqslant P<0{\cdot}05$; **$0{\cdot}001 \leqslant P<0{\cdot}01$; ***$P<0{\cdot}001$. (*) edge of significance: $0{\cdot}05 \leqslant P<0{\cdot}1$.

were taken as exponents in further analysis, along with total biomass per plot as an intermediate.

The MANOVA procedure revealed an overall decrease in frequency, total biomass and chance of flowering of *D. majalis* due to nitrogen and phosphorus application (Table 1, left-hand side). The third order interaction between N, P and K for total orchid biomass was also significant, indicating that when the three nutrients were given together yields were much higher than expected from the main effects and higher order interactions.

### *Orchid performance in relation to yields of co-occurring species*

Total standing crop and the amount of accumulated litter varied between treatments (Fig. 3). Above-ground biomass of the surrounding vegetation increased as a result of nitrogen and potassium fertilization. The amount of litter increased as a result of

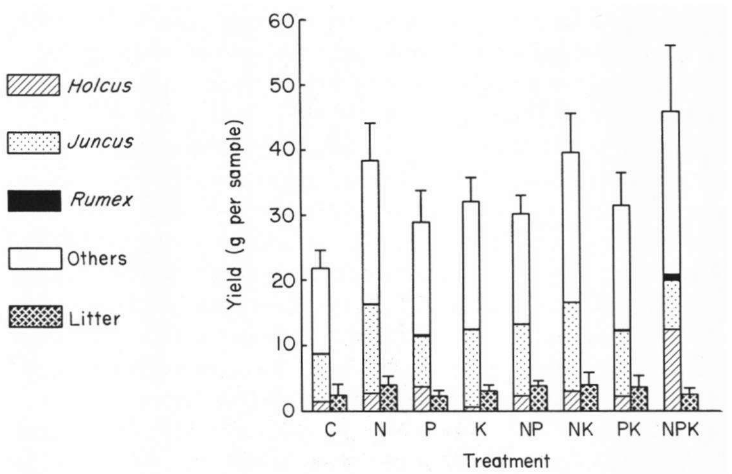

**Fig. 3.** Total above-ground standing crop (left-hand bars) and amount of litter (right-hand bars) in the various fertilization treatments. Values represent the mean of five replicates; error bars indicate standard deviation of the means. The contribution of the most abundant plant species (*Holcus lanatus* and *Juncus acutiflorus*) and of the most important competitors of *Dactylorhiza majalis* (*Holcus lanatus* and *Rumex acetosa*) is shown. Yields are given as g dry weights per 10 × 40 cm vegetation sample.

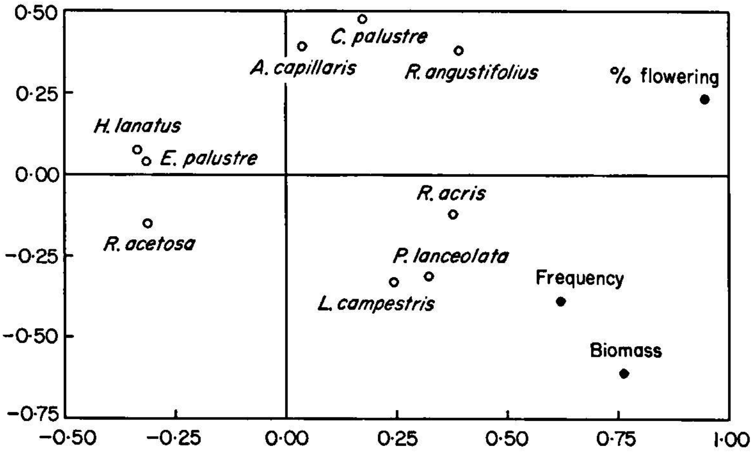

**Fig. 4.** Canonical correlations between number of individuals ('frequency'), total above-ground biomass (in g dry weight m$^{-2}$) and flowering incidence (% flowering; all three performance variables solid dots) of *Dactylorhiza majalis* and plot biomass of co-occurring plant species (open dots). Co-occurring species exhibiting non-significant loadings (<0·30) on the axes have been omitted. For further explanation see text.

nitrogen application, but was smaller than expected from the main effects when N and K were given together. Density, summed above-ground biomass and flowering incidence of *D. majalis* were not correlated to these variables (results not shown).

Orchid performance, however, was correlated to 1991 yields of some of its accompanying plant species (Fig. 4). The main axis calculated in the CCA revealed that all three orchid performance variables were correlated positively to yields of *Rhinanthus angustifolius, Ranunculus acris* and *Plantago lanceolata. Holcus lanatus, Equisetum palustre* and *Rumex acetosa* were negatively correlated with these to about the same extent. Apart from this, orchid frequency and biomass were positively correlated to yields of *Luzula campestris* and *Plantago lanceolata* and adversely to yields of *Cirsium palustre* and *Agrostis capillaris*. Results for *Rhinanthus angustifolius* are hard to interpret, since a negative correlation with orchid yield and frequency on axis 2 is superimposed on a positive one on axis 1. Of these, axis 1 scores are more important, since they explain a larger fraction of variance in the dependent variables (39% of observed variance for the first, and 9% for the second axis). Except for *Cirsium palustre*, canonical correlations were not very strong (between 0·30 and 0·40).

When corrected for yield differences of relevant species as discussed above, significant N- and P-effects could only be demonstrated for frequency of occurrence of the orchid (Table 1, right-hand side). Main nutrient effects on total orchid shoot biomass and flowering incidence disappeared, indicating that they probably originate from increased competition that *D. majalis* experiences from the species mentioned above. The significant NPK interaction for total biomass also disappeared as a result of the correction procedure, while a positive NK interaction on flowering incidence became significant. Examination of untransformed data revealed that this effect was due to a proportionally larger increase in generative plants than in vegetative ones.

*Holcus lanatus* responded positively to nitrogen and phosphorus application. Because yields of *H. lanatus* are negatively correlated to performance of *D. majalis*, negative effects on shoot biomass and chance of flowering of the orchid is probably for the larger part due to increased competition with this species. Apart from this, *H. lanatus* was stimulated by K-fertilization when N or both N and P were added and was especially abundant in NPK-treated plots (Fig. 3). *Rumex acetosa* was amongst others stimulated by P-application; although yields of this species are rather low, it can analogously be expected to play a role in establishing a vegetation-mediated negative P-effect on orchid biomass and flowering incidence. Apart from this, the species was stimulated by K-fertilization, and disproportionally more when given simultaneously with N, P, or both.

The dominant plant species, *Juncus acutiflorus*, showed little response to nutrient application in 1991. Moreover, performance of *D. majalis* was not correlated with biomass of this species.

## CONCLUSIONS

From this study it can be concluded that both nitrogen and phosphate fertilization are detrimental for performance of *D. majalis*. Overall negative effects of addition of the nutrients were found with respect to frequency of occurrence, total shoot biomass per plot and flowering incidence. When variation in orchid performance due to differences in vegetation composition is extracted from overall variance, leaving more direct effects of nutrient applications, results indicate that the mechanism by which the variables are affected by both nutrients is not entirely similar, however. Differences in biomass of associated species, for the greater part due to differences in fertilization regime, indeed account for a part of the variation in orchid performance. For total orchid shoot biomass and proportion of orchid individuals flowering, changes in yields of these species appear to be the primary way by which nutrient effects are expressed. The most prominent of these are *Rumex acetosa* and *Holcus lanatus*, which can be considered important competitors of *D. majalis*. Increased competition with *R. acetosa* seems relevant in determining the orchid's negative P-response, while nitrogen and phosphorus application increase competition with *H. lanatus*. Although global nutrient effects are, of course, always a combination between direct and vegetation-mediated effects, the contribution of the latter seems to be so prominent that they are sufficient to explain the decrease in total orchid yield and flowering rate. Only the negative N- and P-effects of orchid frequency could not be attributed to the nutrient response of competing plant species; the negative impact of these two nutrients on density of the orchid is therefore likely to be more direct.

## DISCUSSION

The experimental site described in this chapter forms the most mature stage in a succession series triggered by cessation of fertilizer application and introduction of annual hay-making in former agricultural grasslands. Glasshouse experiments showed a shift from nitrogen and potassium limitation in early successional stages to simultaneous N-, P- and K-limitation in more mature stages of development, K- and P-limitation becoming gradually more important as the succession proceeded (Olff & Pegtel 1994). N- and K-fertilization in the same plots described here indeed led to higher standing crop. The lack of response of total vegetation biomass to P-addition is probably caused by immobilization of phosphate by precipitation generated by seepage of deep groundwater with high iron contents (Olff & Pegtel 1994). These observations support the findings in this study that changes in performance of *D. majalis* caused by nutrient additions cannot be entirely due to indirect competition effects. Although negative P-effects on *D. majalis* can be attributed rather to increased yields of species like *Holcus lanatus* and *Rumex acetosa* instead of to a significant response of total standing crop of the vegetation, the absence of a negative K-effect would be difficult to explain under the assumption that orchid performance is entirely determined by vegetation response. In the glasshouse experiment, potassium addition increased total yield of the sods even more than nitrogen or phosphate supply, while potassium also stimulated total above-ground biomass of the vegetation in the field.

From results of this study it can be argued that nitrogen is the most relevant factor to explain the decline of *D. majalis* when nutrient availabilities increase, followed by phosphate. Both nutrients may directly affect frequency of the orchid, and bring about vegetation changes that indirectly hamper performance of individuals.

Negative effects of phosphate on growth and occurrence of the orchid can be expected to be a major cause of the restriction of this species to the later stages in this succession series. When comparing vegetation responses 2, 6, 19 and 45 years after change in management, nitrogen could already be shown to be limiting after 2 years, while no substantial increase in N-limitation is evident after 19 years (cf. Olff & Pegtel 1994). The first signs of phosphate limitation, however, were found in fields that were subjected to hay-making without fertilizer application 19 years ago. The extent of phosphate limitation continued to increase towards the most mature stage, which is the experimental site studied here. Since phosphate has a negative effect on frequency of *D. majalis*, phosphate levels may be expected to prevent occurrence of *D. majalis* at least between 6 and 19 years. This period is in concordance with the actual time span that was observed for the orchid to appear in the succession series under restoration management in this system (Bakker & Olff 1994). Appearance of flowering individuals will occur even later in the succession, since probability of flowering and plant size are positively correlated. This was also observed in related species such as *Ophrys apifera* (Wells & Cox 1989) and *Dactylorhiza fuchsii* (Leeson *et al.* 1991).

Differences in time needed for nutrients to become limiting may be accounted for by greater losses of N and K as a result of moving, the build-up of phosphate reserves in roots and the immobilization of phosphate by both soil adsorption and precipitation as calcium and iron salts, favoured by high concentrations of these ions in the seepage water. Phosphate withdrawn from the direct available pool may, however, still affect orchid growth indirectly via their mycorrhizal fungi, which are of paramount importance in their life cycle. Although access to insoluble phosphate is less probable (cf. Harley & Smith 1983), orchid mycorrhizal fungi may alter phosphate availability via increased uptake (Alexander *et al.* 1984) and by biotrophic or necrotrophic degradation of organic matter, which is associated with their parasitic or saprophitic existence.

## ACKNOWLEDGEMENTS

The State Forestry Department (SBB) allowed access to their nature reserve and collection of data. Adriaan van der Veen and Tanja van Vliet performed the laborious sorting of the vegetation samples. Prof. dr. Jelte van Andel corrected earlier versions of this manuscript. Their contributions to this study are gladly acknowledged.

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