University of Groningen

Effects of light and nutrient availability on dry matter and N allocation in six successional grassland species. Testing for resource ratio effects

Oloff, Han

Published in:
Oecologia

DOI:
[10.1007/BF00317420](https://doi.org/10.1007/BF00317420)

IMPORTANT NOTE: You are advised to consult the publisher's version (publisher's PDF) if you wish to cite from it. Please check the document version below.

Document Version
Publisher's PDF, also known as Version of record

Publication date:
1992

[Link to publication in University of Groningen/UMCG research database](#)

Citation for published version (APA):

Oloff, H. (1992). Effects of light and nutrient availability on dry matter and N allocation in six successional grassland species. Testing for resource ratio effects: Testing for resource ratio effects. *Oecologia*, 89(3), 412-421. <https://doi.org/10.1007/BF00317420>

Copyright

Other than for strictly personal use, it is not permitted to download or to forward/distribute the text or part of it without the consent of the author(s) and/or copyright holder(s), unless the work is under an open content license (like Creative Commons).

The publication may also be distributed here under the terms of Article 25fa of the Dutch Copyright Act, indicated by the "Taverne" license. More information can be found on the University of Groningen website: <https://www.rug.nl/library/open-access/self-archiving-pure/taverne-amendment>.

Take-down policy

If you believe that this document breaches copyright please contact us providing details, and we will remove access to the work immediately and investigate your claim.

Downloaded from the University of Groningen/UMCG research database (Pure): <http://www.rug.nl/research/portal>. For technical reasons the number of authors shown on this cover page is limited to 10 maximum.

Effects of light and nutrient availability on dry matter and N allocation in six successional grassland species

Testing for resource ratio effects

Han Olff

Department of Plant Biology, University of Groningen, P.O. Box 14, 9750 AA Haren (Gn.), The Netherlands

Received February 3, 1991 / Accepted in revised form October 12, 1991

Summary. Recent discussions on determinants of competitive success during succession require the study of the combined effect of light and nutrient availability on growth and allocation. These effects can be used to predict the outcome of competition at changing resource availabilities. This work is part of a study on the successional sequence in permanent grassland starting after fertilizer application is stopped, but with continued mowing, in order to restore former species-rich communities. This yields a successional sequence which proceeds from grasslands with a high nutrient availability and a closed canopy, to grasslands with a low nutrient availability and an open canopy. If allocation is related to competitive ability, species from the productive stages would be expected to allocate more biomass and nitrogen to leaves, which could make them better competitors for light, while species from the unproductive stages would allocate more biomass to roots, which could make them better nutrient competitors. This study reports on growth, specific leaf area (SLA), vertical display of leaves, and allocation of biomass and nitrogen of six grassland species from this successional sequence at 16 combinations of light and nutrient supply. Species from the poorer successional stages reached a lower final dry weight than species from the richer stages, over all treatment combinations. The experimental design made it possible to test for unique effects of the resource ratio effect of light and nutrients on allocation characteristics. This resource-ratio effect was defined as the ratio light intensity/(light intensity + nutrient supply rate), using standardized levels for the treatments. The within-species variation (plasticity) in both allocation of dry matter and nitrogen was linearly related to this resource-ratio effect. Some interspecific differences in this relationship were found which could be related to the position of the species along the successional gradient. However, the range of plasticity in allocation pattern expressed within each species was much larger than the differences between species. It was concluded that allocation differences between these grassland species are relatively unimportant, given the large amount of plasticity in these

traits. Interspecific differences in SLA and vertical stature seemed to be more important in explaining the position of species along the successional gradient.

Key words: Allocation – Plasticity – Succession – Resource ratios

Recent literature shows an increasing interest in the mechanisms causing plant species to be separated along gradients of habitat productivity. In general it is found that plant species from infertile soils have a low maximal relative growth rate (Grime and Hunt 1975), allocate more resources to their root systems, have lower tissue nutrient concentrations (Chapin 1980; Poorter 1989; Poorter et al. 1990) and have a longer residence time of nutrients in the plant, which decreases the nutrient demand per unit time (Berendse and Aerts 1987). However, different causal interpretations of these patterns are possible. For instance, species from infertile conditions may grow slowly because this might decrease their demand for nutrients (Grime and Hunt 1975; Chapin 1980). This could make them better ‘stress tolerators’ than rapidly growing species from more productive habitats, which might be better competitors for all resources (Grime 1979). Alternatively, the lower relative growth rate of species from infertile conditions could also be a consequence of the necessity of competing for nutrients when they are in short supply (Tilman 1988). Allocation of biomass and nutrients to roots reduces the allocation to plant parts involved in photosynthesis, thus reducing the maximum relative growth rate, but enabling the plant to reduce nutrients in the soil to lower levels, making them better competitors for nutrients (Tilman 1988).

A second topic over which there is little consensus is the importance of plasticity in allocation patterns. One view is that species from fertile habitats are more plastic in allocating biomass to roots, which allows them to explore patches of sudden high nutrient availabilities (Campbell and Grime 1989; Grime et al. 1986). This

contrasts with the statements made by Tilman (1988) that plasticity in allocation patterns is expected to be smaller than fixed differences between species, and thus will have little effect on the long-term outcome of competition. Furthermore, it is possible that plants are not adapted by differences in (plasticity of) allocation patterns, but that fixed differences between species in morphology and distribution (architecture) within the root or shoot are more important in coping with changing resource availabilities (Grime et al. 1989).

For situations where the vegetation is shifting from nutrient limitation to light limitation, Tilman (1985) proposed his resource-ratio hypothesis of plant succession. This hypothesis states that, for these situations, the outcome of competition will depend on the ratio of light to nutrient availability. The course of changes in species composition would then be determined by changes in this ratio, which usually proceeds from nutrient limitation to light limitation during primary succession. However, effects of different resource ratios on growth and allocation have not yet been studied with regard to this hypothesis. These effects have to be known in order to understand competitive replacement due to changing resource ratios.

The present study reports on an experiment using species from a successional sequence which was initiated by the cessation of fertilizer application to permanent grassland with continued mowing (see Bakker 1989; Olff et al. 1990; Olff and Bakker 1991). The vegetation in our successional series is likely to change in the opposite direction, from light limitation to nutrient limitation. This allows us to determine whether successional changes in the opposite direction can also be predicted by changing resource ratios. The analysis of productivity data over a 17-year period revealed that the sudden reduction in nutrient supply to these grasslands resulted in a gradual decrease in peak standing crop from about $800 \text{ g} \cdot \text{m}^{-2}$ to $300 \text{ g} \cdot \text{m}^{-2}$, an increase in the light availability in the sward and in continuous species replacement (Bakker 1989; Olff and Bakker 1991). Other research suggests that nitrogen is the main soil resource limiting productivity in these grasslands (Pegtel 1987; Olff unpubl.).

In this paper we address the following questions: (i) Can the effect of light and nutrient availability on the distribution of dry matter and nitrogen in the plant be modelled as a linear effect of a resource-ratio effect? (ii) Do species from different successional stages differ in response of these allocation patterns to this resource-ratio effect? (iii) How important are architectural differences between species in explaining their position along the successional gradient?

To address these questions we grew six species from our successional sequence at 16 combinations of light and nutrient supply in a climate chamber, and measured various growth, allocation and architecture characteristics.

Material and methods

Resource ratio effects on allocation

Changing the light intensity or nutrient supply to the plant causes shifts in allocation patterns. This effect has been called the functional equilibrium between roots and shoots (Brouwer 1963). If nitrogen is the main soil resource limiting plant growth, the balance between growth and nitrogen uptake may be described following Davidson (1969) as

$$(dW/dt)(l/W_l) \cdot (W_l/W) = k \cdot (dN/dt)(l/W_r) \cdot (W_r/W) \quad (1)$$

where k is a proportionality constant (see Table 1 for other symbols and abbreviations). Plant growth at a lower light intensity at the same rate of nitrogen supply will lead to a lower total plant biomass than growth at high levels of both resources. Following the principle of balanced growth (Eq. 1), shoot growth will be reduced much less than root growth (or even remain constant). In other words, the reduction in total biomass (W) will be mainly due to a reduction in root biomass (W_r), which will therefore be mainly a function $[W_r(i)]$ of the light supply rate (i). Using the same arguments, it is expected that the leaf biomass (W_l) will be a function $[W_l(r)]$ of the nutrient supply rate (r).

The allocation of dry matter to the leaves can be denoted as

$$W_l/W = W_l/(W_l + W_s + W_r) \quad (2)$$

Incorporating the effects of light and nutrients in Eq. 2 denotes W_l/W as a function of both the light intensity i and the nutrient availability r as

$$W_l/W = f(i, r) = W_l(r)/[W_l(r) + W_s + W_r(i)] \quad (3)$$

So far, we have no information on the exact form of the functions $W_l(r)$ and $W_r(i)$ for our experimental species. Given however that the levels of light and nutrients are chosen below their saturation levels, then Eq. 3 implies that allocation to the leaves (W_l/W) will be expected

Table 1. Abbreviations and units as used in the text

Characteristics	Abbreviation	Unit
Light intensity	i	$\mu\text{Mol} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$
Nutrient supply	r	mM NO_3^- in nutrient solution
Leaf dry weight	W_l	g
Stem dry weight	W_s	g
Root dry weight	W_r	g
Total plant dry weight	W	g
Nitrogen uptake	dN/dt	$\text{g} \cdot \text{day}^{-1}$
Specific leaf area	SLA	$\text{m}^2 \text{ leaf area} \cdot \text{kg leaf dw}^{-1}$
Fraction leaf area higher 4 cm	FLA	—
Leaf N content	N_l	g N
Stem N content	N_s	g N
Root N content	N_r	g N
Total plant nitrogen content	N	g N

Table 2. Factor levels for the combined light and nutrient treatments, with the resulting resource ratio effect R_{ir} .

			Light ($\mu\text{Mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$)			
			65	130	260	520
Coded			1	2	4	8
N supply (mM NO_3^-)	1	1	1/2	2/3	4/5	8/9
	2	2	1/3	1/2	2/3	4/5
	4	4	1/5	1/3	1/2	2/3
	8	8	1/9	1/5	1/3	1/2

to increase proportionally with $r/(r+i)$ or decrease with $i/(r+i)$. Proportional allocation to the roots will increase with $i/(r+i)$ and decrease with $r/(r+i)$. Of course, the term $i/(i+r)$ can only be used when the levels of light and nutrients are standardized (see Table 2), since otherwise the measurement scale used for each factor will influence this ratio. The combined effect of light and nutrients $i/(r+i)$ is called the effect of light to total resource availability (R_{ir} , resource ratio effect) in this paper. It will be tested (i) if the combined effect of light and nutrients on allocation can indeed be summarized as the effect of R_{ir} , and (ii) if species from different stages of the successional series differ in the slope of the regression of W_i/W on R_{ir} , which might reflect interspecific differences in allocation tradeoffs and constraints. A more practical reason for using R_{ir} instead of the more understandable effect i/r is that the latter term responds very asymmetrically to changes in i and r . Using the same level codes, the range of variation in i/r due to variation in i is much larger than variation due to r . Using r/i instead of i/r leads to very different conclusions about its effects, while there is no a priori reason for using the one or the other. This problem does not occur when using $i/(i+r)$, since it is equally sensitive to variation in i and in r .

One problem with the comparison of proportional dry matter allocation between species is that species might develop at different rates along the same relationship of W_i , W_s or W_r with W . This problem is similar to interspecific comparisons of reproductive effort (RE) where RE often depends on vegetative plant biomass (Klinkhamer et al. 1990). These authors proposed the model

$$Y_i = e^a X_i, \quad i = 1, \dots, n \quad (4)$$

or

$$\log Y_i = a + b \log X_i \quad (5)$$

where Y_i is the seed weight of plant i and X_i is the vegetative weight of plant i . For our analysis, we set Y_i to W_i and X_i to $(W_s + W_r)$, to test for the size-dependence of W_i on W . Equations 4 and 5 assume that the relationships have zero intercepts, meaning that leaves or stems without roots are not possible, which is of course a realistic assumption. For $b < 1$, proportional allocation to leaves decreases with plant size, for $b = 1$, allocation to leaves is independent of plant size and for $b > 1$, allocation to leaves increases with plant size. The

same approach was used for testing the dependence of W_s/W and W_r/W on W .

Species and the successional sequence

Six species were chosen for the experiment, differing in their successional position. For each of three successional stages, a representative grass and a forb species were chosen, viz. (from early/productive to late/unproductive) *Lolium perenne* L. – *Anthoxanthum odoratum* L. – *Agrostis canina* L. as perennial grasses and *Rumex acetosa* L. – *Plantago lanceolata* L. – *Succisa pratensis* Moench, as perennial forbs. The generic names will be used from now on.

Lolium and *Rumex* were abundant in the fertilized successional stages, and decreased in abundance after fertilizing was stopped. *Plantago* and *Anthoxanthum* increased during the first 18 years of the successional series, while *Agrostis* and *Succisa* occurred only in fields which had not been fertilized for 30–40 years (Bakker 1989).

Seeds were collected in the experimental area Anloër Diepje, The Netherlands (ref. 53°N, 6°, 40°E) in July 1988 and stored at 5° C until further use.

Experimental procedure

The six species were individually grown from seed at 4 nutrient levels and 4 light intensities in a 4×4 full factorial design with 6 replicates per cell (576 plants). The relative spacings of both experimental factors were chosen as 1, 2, 4 and 8. Combination of both factors resulted therefore in 7 different levels of the resource ratio effect R_{ir} as listed in Table 2. Seeds of each species were sown in petri-dishes and germinated at 25/15° C, 12/12 h light (day/night). Seven days after sowing, the seedlings were transferred to flat containers in an 1:1 mixture of silversand and peat covered with a glass plate and placed in climate chambers at 25° C, 18 h light:6 h dark ($520 \mu\text{mol} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$). At day 17 after sowing, the plants were transferred to 30-cm deep pots (1 plant per pot) containing 4500 g (dw) coarse river sand which had been sieved to a grain size of 0.8 mm. The openings in the bottom of the pots were covered with a fine nylon mesh to prevent loss of sand. The pots were watered daily during the first week and every other day during the rest of the experiment with 1 l of different strengths of a nutrient solution. When fully saturated, the sand contained 0.5 l water and therefore application of 1 l nutrient solution replaced most of the interstitial solution. The nutrient solution at the highest nutrient level (treatment 8) contained 4.67 mM K^+ , 3.00 mM Ca^{2+} , 1.33 mM Mg^{2+} , 8.00 mM NO_3^- , 0.67 mM H_2PO_4^- and 2.33 mM SO_4^{2-} . This solution was diluted 2-, 4- and 8-fold, coded as nutrient treatments 4, 2 and 1, respectively. Each dilution received the same concentration of micronutrients: 0.1 μM Mn, 3 μM Zn, 20 μM B, 0.5 μM Cu, 0.5 μM Mo and 35 μM Fe.

Light in the climate chambers was supplied by Philips fluorescent tubes (15 W) and was $520 \mu\text{mol} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$ at

the highest intensity (light treatment 8), which approximately equals the ambient light intensity in the field on a cloudy day in summer. Lower intensities were induced by removing 50, 75 and 87.5% of the fluorescent tubes (light treatments 4, 2 and 1, respectively). This reduced the light intensity by the same percentages. Every climate chamber received one light intensity and the placement of the pots was randomized every week within each chamber. Since two climate chambers were available, the experiment was performed in two series of two different pairs of light intensities. It was not possible in this design to correct for this series (block) effect. The plants were harvested 45 days after sowing and various characteristics were determined. The leaves were harvested in two parts: all leaves higher than 4 cm (L1) and all leaves lower than 4 cm (L2). The stems/petioles/leaf sheaths were harvested in the same two parts (S1 and S2, respectively). The leaf area of L1 and L2 was determined (A1 and A2, respectively) using a Li-Cor leaf area meter. The total leaf area per plant was then defined as $A = A1 + A2$. The relative allocation of leaf area to higher leaves was then defined as $FLA = A1 / (A1 + A2)$. The specific leaf area (SLA) of each plant was computed as A/L_w ($m^2 \cdot kg^{-1}$). The dry weight of leaf, stem and root fractions was determined after drying for 48 h at 50°C.

Chemical analyses

For the analyses of total nitrogen the leaves, stems and roots were finely ground. Each plant fraction was pooled for each treatment combination, and the total N content was determined in two duplicate samples drawn from the pooled fraction, using a Kjeldahl procedure. Total plant N content for a treatment-species combination was computed by weighing the concentrations of leaves, stems and roots by the mean dry weight of each fraction.

Data analysis

A factorial ANOVA was computed for each dependent variable to test for main effects and interactions. Furthermore, second order response surfaces to light (i) and nutrients (r) were computed for each dependent variable z as $z = ai + br + ci^2 + dr^2 + eir$, where the parameters a , b , c , d and e were fitted by multiple regression. The parameters of this regression model were computed using treatment levels standardized as $(-1, -5/7, -1/7, 1)$ for both experimental factors. The intercept of the regression model then represents the median response to each (combination of) factor(s). Second order response surfaces were computed for each species and subsequently compared using separate effects analysis. For this, the response curves to the factor explaining most variance were graphically compared at the lowest and highest level of the other factor. For the allocation characteristics, it was tested if the variation alternatively could be summarized by the simple linear effect of the resource ratio R_{lr} .

To test the size-dependence of allocation patterns, the dependence of W_l on $(W_s + W_r)$, of W_s on $(W_l + W_r)$ and of W_r on $(W_l + W_s)$ were tested in a log-log linear regression. The slope and intercept for each species were tested for a significant deviation from zero, using a t -test.

Since the data for the N concentration of each plant part were determined for pooled samples and not for each plant separately, those variables involving N concentration and N allocation were not subjected to ANOVA. Spearman's rank correlation coefficients (r_s) were computed for the correlation of the constants of the

Table 3. Factorial analysis of variance of the effect of the light intensity (LI) and nutrient supply rate (NU) on the total dry weight (ln transformed) of six grassland species (SP)

Effect	df	MS	F	% Var
Total dry weight				
Light	3	207.16	1895.74***	62.8
NUtrients	3	8.71	79.68***	2.6
SPecies	5	58.23	532.82***	26.5
LI × NU	9	0.43	3.95***	0.5
LI × SP	15	2.69	24.64***	4.9
NU × SP	15	0.33	3.05***	0.6
LI × NU × SP	45	0.31	2.84***	2.17
Leaf weight ratio (W_l/W)				
Light	3	0.92	328.17***	53.4
NUtrients	3	0.24	85.16***	13.7
SPecies	5	0.10	34.35***	8.1
LI × NU	9	0.02	7.08***	4.0
LI × SP	15	0.04	15.87***	14.6
NU × SP	15	0.01	2.85***	1.8
LI × NU × SP	45	0.01	2.15***	4.4
Petiole weight ratio (W_s/W)				
Light	3	0.06	28.46***	10.4
NUtrients	3	0.09	39.65***	15.0
SPecies	5	0.16	70.51***	40.4
LI × NU	9	0.01	2.79**	3.0
LI × SP	15	0.02	7.27***	14.8
NU × SP	15	0.01	2.52**	3.8
LI × NU × SP	45	0.01	2.32***	12.6
Root weight ratio (W_r/W)				
Light	3	1.43	355.67***	60.0
NUtrients	3	0.62	154.89***	26.1
SPecies	5	0.03	7.31***	1.6
LI × NU	9	0.02	4.61***	2.5
LI × SP	15	0.02	5.76***	4.9
NU × SP	15	0.01	1.76*	0.8
LI × NU × SP	45	0.01	2.00***	4.0
Specific leaf area (SLA)				
Light	3	17596.18	681.16***	49.8
NUtrients	3	104.61	4.05**	0.2
SPecies	5	7099.98	274.84***	30.1
LI × NU	9	45.46	1.76 ^{ns}	0.2
LI × SP	15	844.95	32.71***	13.9
NU × SP	15	96.80	3.75***	1.2
LI × NU × SP	45	92.99	3.60***	4.6

F-values are given with their level of significance (* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$). The percentage of total explained variance was computed for each effect. Other abbreviations: df – degrees of freedom; MS – mean square

regression equations (median response) with the successional position of the species.

Results

Total dry weight

The total dry weight of the plants was significantly affected by all factors (species, light level, nutrient supply) and their interactions (Table 3). However, most variance was explained by the effect of light (62.8%) and differences between species (26.5%). The experimental variation in nutrient supply rate had a much smaller effect

Fig. 1A–D. Response to light intensity of the (ln) plant dry weight at A, C low (1 mM NO_3^-) and B, D high (8 mM NO_3^-) nutrient supply rate, of six species from a successional grassland series. Each point represents the mean of a species at that combination of treatments ($n=6$). Second-order response surfaces were computed using all four light and nutrient levels, and drawn in this figure at the lowest and highest nutrient level. The multiple R^2 of the regression equations is given in Table 4. Meaning of symbols for grasses: $\circ$ — $\circ$ *Lolium perenne*; $\triangle$ — $\triangle$ *Anthoxanthum odoratum*; $\square$ — $\square$ *Agrostis canina*; and for forbs: $\circ$ — $\circ$ *Rumex acetosa*; $\triangle$ — $\triangle$ *Plantago lanceolata*; $\square$ — $\square$ *Succisa pratensis*

on total biomass (2.6%). The second-order response surfaces computed for each species yielded highly significant regression models (Table 4). The mean biomasses of the species were significantly ranked the same way as their order in succession; later (poorer) successional species had a lower total dry weight ($r_s=0.89$, $P<0.05$). The response surfaces of different species were graphically compared using separate effects analysis, by drawing the response to light of each species at the lowest and highest level of nutrient supply rate (Fig. 1). This confirmed the conclusion that the later successional species had in general a lower biomass. The grass species *Lolium* always had, at all treatment combinations, more biomass than *Anthoxanthum* and *Agrostis*, and the forb *Succisa* had at all treatment combinations less biomass than *Rumex* and *Plantago*. Interspecific differences in plasticity were very small and therefore did not confound the order of response (Fig. 1).

Dry matter allocation

Both light and nutrients, as well as their interactions, significantly affected the allocation of dry matter to leaves, stems and roots. The species were significantly different in each allocation parameter, and all interactions between species and experimental factors were significant (Table 3). The allocation of dry matter to leaves (W_l/W) and to roots (W_r/W) was mainly influenced by light (53.4% and 60.0% of total variance explained) while nutrients had a smaller effect on these parameters (13.7% and 26.1%, respectively). The species were mainly different in their dry matter allocation to stems (W_s/W); the differences between species explained 40.4% of the variance in this trait. For all traits, the species $\times$ light interaction was the most important higher-order effect. The computation of response-surfaces for the response of W_l/W and W_r/W yielded highly significant regression

Table 4. Correlations for the dependence of various plant characteristics on the light intensity and nutrient supply, using a second-order response surface

Trait	Species					
	Lolium	Rumex	Anthoxan	Plantago	Agrostis	Succisa
Total dry weight ^a	0.95***	0.85***	0.94***	0.94***	0.94***	0.64***
Leaf weight ratio (W_l/W)	0.80***	0.69***	0.69***	0.68***	0.79***	0.22*
Stem weight ratio (W_s/W)	0.40***	0.59***	0.40***	0.14*	0.30***	0.30***
Root weight ratio (W_r/W)	0.76***	0.78***	0.67***	0.68***	0.73***	0.64***
Specific leaf area (SLA)	0.88***	0.78***	0.83***	0.83***	0.36***	0.70***
Fraction leaf area > 4 m (FLA)	0.51***	0.24***	0.72***	0.73***	0.35***	—
Fraction total N in leaves (N_l/N)	0.87***	0.87***	0.78**	0.90***	0.81**	0.67*
Fraction total N in stems (N_s/N)	0.70*	0.66*	0.75**	0.25 ^{ns}	0.45 ^{ns}	0.28 ^{ns}
Fraction total N in roots (N_r/N)	0.86***	0.94***	0.87***	0.85***	0.93***	0.79**
Leaf N concentration (N_l/W_l)	0.93***	0.83**	0.95***	0.89***	0.91***	0.93***
Stem N concentration (N_s/W_s)	0.71***	0.80***	0.97***	0.85***	0.89***	0.65*
Root N concentration (N_r/W_r)	0.94***	0.92***	0.88***	0.94***	0.90***	0.90***
Total plant N concentration (N/W)	0.97***	0.93***	0.96***	0.93***	0.96***	0.93***

^a Ln transformed prior to the computations

— No data

The R^2 is given for each regression, with the level of significance (*** $P<0.001$; ** $P<0.01$; * $P<0.05$; ns, not significant)

Table 5. Linear correlations (R^2) for the dependence of various dry matter and N allocation characteristics on the resource ratio effect R_{ir} (see Table 2)

Trait	Species					
	<i>Lolium</i>	<i>Rumex</i>	<i>Anthoxan</i>	<i>Plantago</i>	<i>Agrostis</i>	<i>Succisa</i>
Leaf weight ratio (W_l/W)	0.66***	0.67***	0.55***	0.58**	0.50***	0.14**
Stem weight ratio (W_s/W)	0.28***	0.50***	0.17***	0.07*	0.12***	0.18***
Root weight ratio (W_r/W)	0.63***	0.76***	0.63***	0.59***	0.59***	0.59***
Fraction of total N in leaves (N_l/N)	0.59***	0.81***	0.69***	0.74***	0.58***	0.64***
Fraction of total N in stems (N_s/N)	0.01 ^{ns}	0.58***	0.50***	0.01 ^{ns}	0.01 ^{ns}	0.17 ^{ns}
Fraction of total N in roots (N_r/N)	0.80***	0.89***	0.85***	0.71***	0.86***	0.75***
Leaf N concentration (N_l/W_l)	0.80***	0.70***	0.86***	0.90***	0.82***	0.58***
Stem N concentration (N_s/W_s)	0.51**	0.55***	0.69***	0.86***	0.61***	0.18 ^{ns}
Root N concentration (N_r/W_r)	0.67***	0.80***	0.63***	0.75***	0.56***	0.59***
Total plant N concentration (N/W)	0.85***	0.86***	0.89***	0.92***	0.72***	0.63***

Table 6. Parameters ($\pm$ SE) of the linear regression of leaf dry weight (W_l), stem dry weight (W_s), root dry weight (W_r) with the remaining plant dry weight, for assessing effects of total plant size on dry matter allocation (see text)

Species	$\log(W_l)$ vs. $\log(W_s + W_r)$		W_s vs. $W_l + W_r$		W_r vs. $W_l + W_s$	
	Slope	Intercept	Slope	Intercept	Slope	Intercept
<i>Lolium perenne</i>	0.77 ± 0.02	-1.02 ± 0.11	0.99 ± 0.03	-1.30 ± 0.16	1.23 ± 0.05	-1.94 ± 0.26
<i>Anthoxanthum odoratum</i>	0.85 ± 0.04	-0.44 ± 0.22	0.81 ± 0.04	-0.34 ± 0.23	1.07 ± 0.07	-0.86 ± 0.38
<i>Agrostis canina</i>	0.74 ± 0.02	-1.07 ± 0.11	0.97 ± 0.04	-1.40 ± 0.20	1.25 ± 0.05	-1.80 ± 0.25
<i>Rumex acetosa</i>	0.80 ± 0.03	-0.86 ± 0.16	0.90 ± 0.03	-1.13 ± 0.18	1.18 ± 0.05	-1.44 ± 0.29
<i>Plantago lanceolata</i>	0.69 ± 0.02	-0.86 ± 0.09	0.97 ± 0.03	-0.84 ± 0.15	1.33 ± 0.05	-2.24 ± 0.23
<i>Succisa pratensis</i>	1.00 ± 0.05	-0.25 ± 0.16	0.62 ± 0.04	-0.06 ± 0.16	1.17 ± 0.05	-1.19 ± 0.18

Data (mg) were used, and natural-log transformed prior to the analysis. All regressions were significant ($P < 0.001$), and all parameters were significantly different from zero ($P < 0.05$, t -test)

equations (Table 4). Decreased light availability promoted allocation to leaves, while decreased nutrient availability promoted allocation to roots. These opposite effects resulted in a good correlation with the resource ratio R_{ir} , which explained nearly as much variance in a much simpler model, and therefore showed much higher F values than the second-order response surface (Tables 4 and 5; Fig. 2; F values not shown). This implies that the resource ratio effect R_{ir} was an appropriate summary of the effect of light and nutrients on the distribution of dry matter between leaves and roots. Since the variation in W_s/W could be mainly ascribed to species differences (Table 3), the effect of R_{ir} on allocation to stems was much smaller (Table 5). The standardized mean response for the linear model represents the mean response over all treatments where the relative availability of light and nutrients was the same, and only the level of each was varied simultaneously (treatments 1,1), (2,2), (4,4) and (8,8)). It was found that the mean responses of the allocation parameters W_l/W and W_s/W were very similar for all the species, and hence not related to the species order in the successional sequence (Fig. 2). An exception to these patterns was *Succisa*, which was less plastic in all allocation traits than the other species, and allocated more biomass to roots (Fig. 2). No significant rank correlation was found between the amount of plasticity in dry matter allocation

Fig. 2A–F. Response of dry matter allocation of six successional grassland species to the resource ratio effect R_{ir} , defined as (light intensity/(light intensity + nutrient supply rate)) (see Table 2). The response of each species was averaged for each level of R_{ir} . For each species, the allocation to leaves (circles), to stems/leaf sheaths (triangles) and to roots (squares) is given at each level of R_{ir} . The R^2 of the regression equations, with level of significance, is given in Table 5

(slope of the linear regression equation) and the successional position of the six species. Due to the opposite effect of light and nutrients on allocation to shoots and roots, R_{lr} showed a negative correlation with W_l/W and W_s/W , and a positive correlation with W_r/W (Fig. 2).

The allocation of dry matter to stems (W_s/W) was much less influenced by light and nutrient availability than allocation to leaves and roots, for both regression models (Table 4, Fig. 2). For the linear model, the allocation of dry matter to stems was much less variable than allocation to leaves and roots (Fig. 2). The only species differing in its mean response was *Agrostis*, which allocated much more to stems over all ratios than the other species (Fig. 2).

The regression analysis where the ln-transformed biomass of leaves, stems and roots were each regressed against the remaining plant dry weight revealed that in all species the allocation to leaves and stems decreased at higher total plant dry weight (slope < 1), while the allocation to roots increased (slope > 1), when averaged over all treatments (Table 6). *Succisa* was the only species which clearly differed from the others in all parameters, indicating that the allocation differences in this species were due to a different relationship of allocation with total dry weight. The higher allocation of *Agrostis* to stems was not found in this analysis, indicating that this species developed at a different rate along the same relation of W_s with W (Table 6).

Distribution within the shoot

The variance in specific leaf area (SLA) could mainly be ascribed to the effect of light (49.8%), differences between species (30.1%) and by the interaction between these two effects (13.9%, Table 3). Nutrients had little effect on the SLA and no interaction between light and nutrients was found (Table 3). The second order regression model explained most variance in SLA, except for *Agrostis* which was less plastic in SLA (Table 3, Fig. 3). The rank correlation between the mean response of different species and their successional position was not significant. However, the large light $\times$ species interaction

Fig. 3A–D. Response to light intensity of the specific leaf area (SLA) at A, C low successional grassland series. The R^2 of each regression equation, with level of significance, is given in Table 4. See Fig. 1 for meaning of symbols and lines

Fig. 4A–D. Response to light intensity of the fraction of leaf area higher than 4 cm (FLA) at A, C low (1 mM NO_3^-) and B, D high (8 mM NO_3^-) nutrient supply rate, of six species from a successional grassland series. The R^2 of each regression equation, with level of significance, is given in Table 4. See Fig. 1 for meaning of symbols and lines

from the ANOVA indicated large interspecific differences in plasticity of the SLA in response to light. The species from the earlier successional stages appeared to be more plastic in response to light than the late species (Table 3, Fig. 3). The main difference was that the early successional species increased their SLA at lower light intensities more than the later species did, both at low and high nutrient availability (Fig. 3).

The fraction of the leaf area displayed higher than 4 cm (FLA) was not subjected to ANOVA because *Succisa* had always zero values for this trait. The FLA was mainly determined by light (Fig. 4). A significant negative rank correlation between the mean response and the successional position was found ($= -0.89$, $P < 0.05$), indicating that early successional species displayed their leaf area higher (were taller) than late successional species (Fig. 4). The species were also very different in the amount of plasticity in this trait. *Lolium* and *Rumex* always displayed most leaf area higher than 4 cm, whereas *Agrostis* and *Succisa* were always prostrate (Fig. 4). The successional intermediate species *Anthoxanthum* and *Plantago* were the most plastic in this trait (Fig. 4).

Nitrogen concentration

The nitrogen concentration of the leaf, stem and root fractions was well described by the second-order response surfaces to light and nutrients, which explained up to 97% of the variance (Table 4). However it should be noted that no replicate data were obtained for each treatment combination, and that therefore the F values and R^2 are based on fewer degrees of freedom than for the dry weight variables (11 and 91 df for the error term, respectively). The N concentration in leaves, stems and roots increased with the nutrient supply rate, and decreased with the light intensity. The opposite effects of light and nutrients on the N concentration could be summarized as a linear effect of the resource ratio effect R_{lr} (Table 5). The R^2 values were somewhat lower than for the second-order regression model, but the F values were much higher, due to fewer degrees of freedom (re-

Fig. 5A-F. Response of the nitrogen concentration of leaves, stems and roots in six grassland species to the resource ratio effect R_{ir} . Symbols as Fig. 2

Fig. 6A-F. Relationship between the leaf N concentration and the root N concentration in six successional grassland species. The correlation coefficient and slope of the regression equation is given for each species

sults not shown). The forb species were very different in the mean response to the resource ratio effect (Fig. 5). *Rumex* had a high N concentration (per unit dry weight) in the leaves, while *Plantago* had a high N concentration in the leaves and stems. *Succisa* had a much lower N concentration of all plant parts. The N concentration of the different plant parts of *Succisa* was also the least variable. The interspecific differences between forbs in leaf N concentration were larger than differences in stem and root N concentration (Fig. 5). The grasses responded much more similar to R_{ir} (Fig. 5).

In none of the plant parts was a consistent relationship found between successional position and plasticity in N concentration. Since the N concentrations in the roots and leaves were similarly affected by light and nutrients, both variables were closely correlated in each species (Fig. 6). This implies that the concentration of

N in the different plant parts can be summarized into one variable, the total plant N concentration (N/W). This variable showed a good correlation with R_{ir} , and reflected mainly the differences between species as discussed for the separate plant parts (Table 5).

Allocation of nitrogen

Allocation patterns of nitrogen in the plant can be viewed as the combined effect of dry matter allocation and the N concentration of each plant part. Since the N concentration was not determined at the individual plant level, the results were directly subjected to regression analysis. The allocation of N to leaves (N_l/N) and roots (N_r/N) was well described by the second order regression models (Table 4). Light and nutrients had an opposite effect on nitrogen allocation; decreasing the light intensity increased the allocation of N to the leaves, while decreasing the nutrient availability increased the allocation of N to the roots. The R^2 of the regression models were not significant for N_s/N in three species and low but significant in the other three species (Table 4), indicating that light and nutrients mainly affected the distribution of nitrogen between leaves and roots. The opposite effect of light and nutrients could be summarized as highly significant effects of the resource ratio R_{ir} on N allocation to leaves and roots (Table 5), with higher F values than the second order response surfaces (F values not shown). Examination of differences between species revealed that *Succisa* allocated more N to roots, while *Agrostis* allocated more N to stems (Fig. 7). This difference in *Succisa* seemed to be due to a different morphological development along the relation of W_r with W , while in *Agrostis* this relationship was the same as in the other species. From the effect of R_{ir} , it could furthermore be concluded that the largest effects of light and nutrients were on the N distribution

Fig. 7A-F. Response of the allocation of nitrogen to leaves (circles), stems (triangles) and roots (squares) of six grassland species to the resource ratio effect R_{ir} . Symbols as Fig. 2

between leaves and roots, while the allocation to stems was much less affected (Fig. 7).

Discussion

This study revealed several differences between species from the early, fertilized successional stages and species from later, unfertilized stages. The species from the later stages had a lower total dry weight, and therefore probably a lower relative growth rate. Several important components of growth rate can be discussed. The interspecific differences in the allocation of dry matter and nitrogen to leaves and roots were relatively small (8.1% and 1.6% of total variance explained for dry matter allocation to leaves and roots, respectively). The effects of light and nutrients on allocation within each species were much larger. Nitrogen and dry matter allocation were similarly affected by light and nutrients: more allocation to leaves at low light/high nutrients and more allocation to roots at low nutrients/high light. Over all treatments, *Agrostis* allocated more to stems than other species did, while *Succisa* allocated more to roots. The allocation of dry matter and N was found to be linearly related to the resource ratio R_{lr} , and all species except *Succisa* showed a large plasticity in allocation pattern in response to R_{lr} .

The main effect of variation in light and nutrient supply in this experiment was the redistribution of dry matter and N between leaves and roots, while the allocation to stems was remarkably constant at different values of R_{lr} . However, given the small differences between species in allocation pattern, and the large plasticity of these patterns, it does not seem that fixed differences in allocation patterns between species are the main explanation for the observed order of the species in our successional series. The same conclusion was reached by Olff et al. (1990) in a smaller experiment and by Berendse and Elberse (1989), using different grassland species from a comparable successional sequence.

The interspecific differences in specific leaf area (SLA) and fraction of leaf area >4 cm (FLA) were much larger in our experiment. *Lolium* and *Rumex* increased their SLA at lower intensities, while later successional species were much less plastic in this trait. Producing more leaf area with the same resource investment in leaves can produce a growth advantage under light limiting conditions (Givnish 1988; Rice and Bazzaz 1989), as occurring early in succession in fertilized grasslands. Furthermore, the early successional species displayed more of their leaf area in the higher regions, which might yield a growth advantage under conditions of high biomass and a closed canopy in the field (Bobbink et al. 1988).

Several remarks can be made on the observation that our successional grassland species showed only very small interspecific differences in allocation to leaves and roots in the present experiment, contrary to the predictions of Tilman (1985), while the plasticity in these traits was very large.

Firstly, the allocation pattern which is observed in a plant in the field at a given moment of time is not

only determined by the rate at which a plant allocates resources to each plant part, but also by differences in the rate of losses of carbon and nutrients from the different plant parts. In fact, Berendse et al. (1987) and Aerts (1989) concluded that the outcome of competition between an evergreen shrub and a deciduous grass at low nutrient availability was mainly determined by the interspecific differences in the residence time of nitrogen. In our present short-term growth experiment it was not possible to study allocation differences between species caused by differences in loss rate of dry matter or nitrogen between plant parts. When the turnover rate of some plant parts is very different between species, then interspecific allocation differences will increase with time. In the seedling stage, plants may allocate their resources in a way that maximizes their relative growth rate, within their physiological and morphological limits to plasticity. The resulting allocation patterns might show less variation, because they are highly constrained by the physiological trade-offs described earlier. It may be argued therefore that interspecific differences in allocation patterns are likely to increase with plant age, and had not yet been expressed in the present experiment. This point should be investigated in longer term experiments.

A second possible explanation for the observation that allocation differences between the species in our experiment were of minor importance compared to their plasticity, could be that interspecific differences in allocation patterns never become important determinants of competitive success during this successional sequence. The differences between species in distribution of dry matter within the shoot (SLA and FLA) appeared to be a better explanation for their successional position. A high SLA and an erect stature might confer a competitive advantage when the light profile in the vegetation shows a steep gradient, as in productive situations. Displaying the leaves in the upper part of a hay-field or pasture canopy means that a major part of the living plant C and N pool is removed annually. This might not be a problem for the plant at high levels of nutrient availability, since competition in these situations is expected to be mainly for light. At low nutrient availability however, yearly removal of a major part of the shoot every year might confer a disadvantage in competition for nutrients (Berendse 1985; Tilman 1988).

A third possible explanation of why the allocation patterns seem less important in our situation is that grassland subjected to cutting and grazing experience very high overall loss rates of biomass. This prevents species with a long above-ground residence time of nutrients from becoming dominant, and might therefore constrain all species in similar average allocation patterns but with a large plasticity (Berendse and Elberse 1989). We have to keep in mind that an inherent feature of permanent grassland is that a major part of the above-ground biomass is removed every year. This means that species have to cope with large loss rates of biomass and nutrients, which prevents species that have permanent above-ground structures, such as shrubs and trees, from invading the vegetation. The best competitors in this situation will not be the species which can, in the

long run, reduce resources to the lowest level (*sensu* Tilman 1988), but those which can capture the resources first within a season. The importance of plasticity for competitive success might decrease with productivity during succession. This makes grassland succession with yearly mowing very different from most other primary and secondary succession series, and might imply that species replacement is mediated by quite other mechanisms.

Which interactions between plant characteristics and resource availabilities are ultimately directing successional dynamics in grasslands with mowing will be studied further in long term competition experiments, and by monitoring temporal changes in the availability of light and nutrients in the field.

Acknowledgements. The investigations were supported by the Foundation for Biological Research (BION), which is subsidized by the Netherlands Organization for Scientific Research (NWO). I am grateful to Jelte van Andel, David Tilman, Hans Lambers, Hendrik Poorter, David Wedin, Nancy Johnson and the members of our Plant Ecology group for their valuable comments on an earlier draft, and to Jacob Hogendorf and Sieze Nijdam for technical assistance.

References

- Aerts R (1989) Nitrogen use efficiency in relation to nitrogen availability and plant community composition. In: Lambers H et al. (eds) Causes and consequences of variation in growth rate and productivity of higher plants. SPB Academic, The Hague, The Netherlands, pp 285–297
- Bakker JP (1989) Nature management by cutting and grazing. Junk, Dordrecht, The Netherlands
- Berendse F (1985) The effect of grazing on the outcome of competition between plants with different nutrient requirements. *Oikos* 44:35–39
- Berendse F, Aerts R (1987) Nitrogen-use-efficiency: a biological meaningful definition? *Funct Ecol* 1:293–296
- Berendse F, Elberse WTh (1989) Competition and nutrient losses from the plant. In: Lambers H et al. (eds) Causes and consequences of variation in growth rate and productivity of higher plants. SPB Academic, The Hague, The Netherlands, pp 269–284
- Berendse F, Oudhof H, Bol J (1987) A comparative study on nutrient cycling in wet heathland ecosystems. 1. Litter production and nutrient loss from the plant. *Oecologia* 74:174–184
- Bobbink R, Bik L, Willems JH (1988) Effects of nitrogen fertilization on vegetation structure and dominance of *Brachypodium pinnatum* L. Beauv in chalk grassland. *Acta Bot Neerl* 37:231–242
- Brouwer R (1963) Some aspects of the equilibrium between over-ground and underground parts. *Jaarb IBS* 213:31–39
- Campbell BD, Grime JP (1989) A comparative study of plant responsiveness to the duration of episodes of mineral nutrient enrichment. *New Phytol* 112:261–267
- Chapin FS (1980) The mineral nutrition of wild plants. *Annu Rev Ecol Syst* 11:233–260
- Davidson RL (1969) Effects of soil nutrients and moisture on root/shoot ratios in *Lolium perenne* L. and *Trifolium repens* L. *Ann Bot* 33:571–577
- Givnish TJ (1988) Adaptation to sun and shade: a whole plant perspective. *Aust J Plant Physiol* 15:63–92
- Grime JP (1979) Plant strategies and vegetation processes. Wiley & Sons, Chichester
- Grime JP, Hunt R (1975) Relative growth rate: its range and adaptive significance in a local flora. *J Ecol* 63:393–422
- Grime JP, Crick JC, Rincon JE (1986) The ecological significance of plasticity. *J Exp Biol* 126:5–29
- Grime JP, Campbell BD, Mackey JML, Crick JC (1989) Root plasticity, nitrogen capture and competitive ability. In: Atkinson D (ed) Plant root systems: their effect on ecosystem composition and structure. Blackwell, Oxford
- Klinkhamer PGL, de Jong TJ, Meelis E (1990) How to test for proportionality in the reproductive effort of plants. *Am Nat* 135:291–300
- Olf H, Bakker JP (1991) Long term dynamics of standing crop, vegetation composition and species richness after the cessation of fertilizer application to hay-fields. *J Appl Ecol* (in press)
- Olf H, Van Andel J, Bakker JP (1990) Biomass and shoot:root allocation of five species from a grassland succession series at different combinations of light and nutrient supply. *Funct Ecol* 4:193–200
- Pegtel DM (1987) Soil fertility and the composition of semi-natural grassland. In: Van Andel J, Bakker JP, Snaydon RW (eds) Disturbance in Grasslands. Junk, Dordrecht, The Netherlands, pp 51–66
- Poorter H (1989) Interspecific variation in growth rate: on ecological causes and physiological consequences. In: Lambers H et al. (eds) Causes and consequences of variation in growth rate and productivity of higher plants. SPB Academic, The Hague, The Netherlands, pp 45–68
- Poorter H, Remkes C, Lambers H (1990) Carbon and nitrogen economy of 24 wild species differing in relative growth rate. *Plant Physiol* 94:612–627
- Rice SA, Bazzaz FA (1989) Growth consequences of plasticity of plant traits in response to light conditions. *Oecologia* 78:508–512
- Tilman D (1985) The resource-ratio hypothesis of plant succession. *Am Nat* 125:827–852
- Tilman D (1988) Plant strategies and the dynamics and structure of plant communities. Princeton University Press, Princeton