The interplay between shifts in biomass allocation and costs of reproduction in four grassland perennials under simulated successional change

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Abstract
When perennial herbs face the risk of being outcompeted in the course of succession, they are hypothesized to either increase their biomass allocation to flowers and seeds or to invest more in vegetative growth. We test these hypotheses in a three-year garden experiment with four perennials (*Hypochaeris radicata*, *Cirsium dissectum*, *Succisa pratensis* and *Centaurea jacea*) by growing them in the midst of a tall tussock-forming grass (*Molinia caerulea*) that may successionaly replace them in their natural habitat. In all species except for the short-lived *H. radicata*, costs of sexual reproduction were significant over the three years, since continuous bud removal enhanced total biomass or rosette number. To mimic succession we added nutrients, which resulted in tripled grass biomass and higher death rates in the shorter-lived species. The simulated succession resulted also in a number of coupled growth responses in the survivors: both enhanced plant size as well as elevated seed production. The latter was partly due to larger plant sizes, but mostly due to higher reproductive allocation, which in turn could be partly explained by lower relative somatic costs and by lower root-shoot ratios in the high nutrient plots. Our results suggest that perennial plants can increase both their persistence and their colonization ability by simultaneously increasing their vegetative size and reproductive allocation in response to enhanced competition and nutrient influxes. These responses can be very important for the survival of a species in a metapopulation context.

Keywords: costs of reproduction, root-shoot ratio, sexual reproductive allocation, succession.

Introduction
On the time scale of succession, plant populations are ephemeral, and will eventually go extinct (Tilman 1987; Falinska 1991): early-successional plant species in grasslands are gradually outcompeted by taller competitors that accumulate biomass (Berendse et al. 1992; Roem and Berendse 2000). In European grasslands high levels of atmospheric deposition of nitrogen enhance the succession process (Crawley 1997). Metapopulation theory predicts that species can lower their regional extinction risks by increasing the rate of colonization of unoccupied habitats by increasing seed
production and dispersal, or by increasing local persistence by adjusting its life history to the changing habitat (Ronce et al. 2005). Since microsites for seedling establishment are often limiting in late-successional grasslands (Kupferschmid et al. 2000), plants can extend their local persistence by increasing their size through vegetative and clonal growth to increase competitiveness and to reduce individual mortality rates. Alternatively, plants may enhance flowering and fruiting, and hence increase the chance to escape to new habitats, which may only be possible at the cost of reduced growth and survival (Abrahamson 1980). Whether and how individual plants are able to alter their life history as succession proceeds is yet unclear. Here we study these life history responses experimentally by subjecting four perennial grassland species to simulated successional change.

Underlying these hypotheses on adaptive biomass allocation is a trade-off between seed production and vegetative reproduction. Costs of sexual reproduction, i.e. any reduction in fitness parameters like survival, growth, plant size or future reproduction due to biomass investment in sexual reproduction, are a crucial element explaining these alternative hypotheses because in the absence of costs plants could change different life history functions independently. Recent studies have shown that trade-offs can be masked by the size-dependent relationship between plant size and investment into e.g. sexual reproduction (Ågren and Willson 1994; Reznick et al. 2000; Ehrlén and van Groenendael 2001). But when plant size is accounted for, trade-offs between life history functions can be found (Méndez and Obeso 1993; Primack and Stacy 1998; Obeso 2002; Hartemink et al. 2004). Therefore we tested for costs of sexual reproduction in this study. We use the method of flower bud removal (Obeso 2002) to investigate if plants switch to increased size, storage or vegetative offspring number when flowering and seed set are inhibited.

The primary aim in our three-year garden experiment was to test the allocation responses to simulated successional replacement in four perennial herbs. Plants can change their reproductive biomass by changing their biomass allocation (i.e. proportional investment) to sexual reproduction, by changing their overall size, or by a combination of size and allocation changes. Only size-independent shifts in allocation or changes in allometric relationships (Sugiyama and Bazzaz 1998) can be regarded as integrated plastic responses of the allocation pattern to changing conditions (Müller et al. 2000; Weiner 2004). In our experiment the target herbs competed with a dominant, tall grass with which they naturally co-occur in nutrient-poor grasslands. We fertilized half of the plots to mimic the accumulation of plant biomass and available nutrients during natural succession with high atmospheric deposition such as in the Netherlands. We explored several mechanisms that may have altered reproductive biomass in the fertilized plots: changes in plant size, in root-shoot ratio and in costs of reproduction.

Material and methods

Costs of sexual reproduction and shifts in allocation patterns were investigated in a three-year (2000-2002) garden experiment with four perennials: *Hypochaeris radicata*, *Cirsium dissectum*, *Succisa pratensis* and *Centaurea jacea*. Allocation of biomass to four parts of the plants was studied: sexual reproductive structures (flower heads, seeds, and buds of flower heads), vegetative plant parts (stems, stem leaves, rosette leaves, and roots), storage organs (the caudex, i.e. the persistent rootstock to which the rosette leaves, stems and roots are attached) and clonal organs (only in *C. dissectum*: rhizomes). Although multiple definitions of reproductive structures exist, we chose to consider only the flower heads as reproductive (and not the stems),
because we determined only the costs of the production flowers and seeds (see treatments).

Study species

*Hypochaeris radicata* L. (Asteraceae) is a relatively short-lived perennial. Its leafless flowering stalks and new rosettes are formed clonally by branching of the taproot (de Kroon et al. 1987; Jongejans and de Kroon 2005). Flowering starts in June and continues until autumn.

*Cirsium dissectum* (L.) Hill (Asteraceae) is a rhizome-forming clonal plant, with monocarpic rosettes. Normally one flower head is formed in June (Jongejans et al. 2005). In the Netherlands *C. dissectum* is a rare and endangered species (Red List 2; van der Meijden 1996) due to the decline of its habitat, nutrient-poor moist grasslands (Lucassen et al. 2003).

*Succisa pratensis* Moench (Dipsacaceae) rosettes are polycarpic and can survive for many years (Adams 1955; Jongejans and de Kroon 2005). New rosettes and up to four flowering stalks emerge laterally from the caudex. Flowering varies from July to September.

*Centaurea jacea* L. s.l. (Asteraceae) is a relatively long-lived perennial, although it has monocarpic shoots: during and after flowering, vegetative side-rosettes are formed on the woody rootstock and appear at the soil surface alongside the flowering stem (Hartemink et al. 2004). Subsequently these rosettes can form new stems that grow horizontally for several centimeters before growing vertically to flower (from June until autumn). The four target species are characteristic for grasslands as they disappear along succession towards taller forbs and woody species. All four species have composite flower heads. Only the seeds of *H. radicata* and *C. dissectum* are plumed and adapted to dispersal by wind (Soons and Heil 2002).

*Molinia caerulea* (L.) Moench (Poaceae) is a tussock forming, tall grass, which occurs in nutrient-poor grasslands and grass heaths. *Molinia caerulea* starts to dominate when nutrient deposition is high (Berendse and Aerts 1984; Aerts et al. 1990), especially when fields are abandoned.

Plant material

Seeds of the four target species were collected in 1998 in the nature reserve ‘Bennekomse Meent’, a nutrient-poor grassland near Wageningen in the Netherlands (52°01'N, 5°36'E; van der Hoek et al. 2004). Cuttings of *M. caerulea* were collected at the same locality. Plants of *C. jacea, S. pratensis* and *C. dissectum* were grown from seed in a greenhouse one year before the start of the experiment. In May 2000 newly formed rosettes of these plants were carefully broken off. For *H. radicata* two-months-old seedlings were used. In order to allow for direct comparisons between the bud removal treatment (see below) and the undisturbed plants, all cuttings and seedlings were grouped in pairs of similar initial size, and of the same genetic identity, or, in case of *H. radicata*, grown from seeds of the same mother plant. One plant of each pair was assigned to the bud removal treatment, the other to the untreated group. Each pair of plants was either assigned to the nutrient addition treatment or to the low nutrients treatment. The size-dependency of allocation in an allometric framework was taken into account by starting the experiment with a range of plant sizes rather than selecting for equally sized plants. The cuttings and seedlings were transplanted into an experimental garden of Wageningen University.

The 320 plants were randomly placed in a randomized block design: four species * two bud removal treatments * two resource treatments * 20 replicates (Fig.
S1). The interval between the target plants was 50 cm. Around each target plant six 
*M. caerulea* clumps of four shoots each were placed in a hexagon with sides of 10 cm. 
Lawn edging was placed 10 cm deep in a circle of 50 cm in diameter around the *C. 
dissectum* plants to prevent this clonally spreading species from growing through the 
whole garden. Measurements on 20 additional plots indicated that these circles of 
lawn edging did not affect the biomass increments of *M. caerulea* (data not shown).

*Treatments*

Nutrient enrichment was applied to half of the plants in the second and third year, 
allowing the plants to establish under the same conditions in the first year of the 
experiment. Nutrient solution was applied to a circular area of 50 cm diameter around 
the target plants. The Hoagland’s stock solution contained KNO₃, Ca(NO₃)₂, MgSO₄, 
and NH₄H₂PO₄ (Gamborg and Wetter 1975). The solution was applied in three 
portions within two months at the beginning of the growing season and was 
equivalent to 120 kg N ha⁻¹ yr⁻¹, which is about three times the atmospheric deposition 
in Dutch agricultural landscapes (Bobbink et al. 1998; van Oene et al. 1999). Van der 
Hoek et al. (2004) found significant shifts in the vegetation composition in the field 
when they applied 200 kg N ha⁻¹ yr⁻¹. In each of the 20 blocks all plants that were 
assigned to the high nutrient treatment were placed together and surrounded with 25 
cm-deep lawn edging to prevent nutrient leakage to the plants of the low nutrients 
group (Fig. S1). The unfertilized plants of each block were also grouped and enclosed 
by the same lawn edging.

Flower buds on flowering stalks were removed three times each month 
throughout the three years of the experiment. Half of the plants received this 
treatment; the other half was allowed to flower and set seeds naturally.

*Measurements*

In addition to monitoring the survival of the target plants, we measured plant size at 
the end of each of the three growing seasons. Rosettes and flower heads were counted. 
Throughout the experiment random flower heads were bagged after flowering to 
estimate average weight of the sexual reproductive tissue: flower head, flowers and 
seeds. Total flower head weight per plant was calculated by multiplying the average 
flower head weight of each species and treatment combination by the flower head 
count of the individual. In September 2002 all plants were harvested. Belowground 
parts were harvested in a circular area of 40 cm diameter around the center of the *M. 
caerulea* hexagon. The plots were dug out at least 25 cm deep and only a very small 
percentage of the fine roots were lost as the plants rooted shallowly in the sandy soil. 
The roots of the target plant and the grass were relatively easy to separate since *M. 
caerulea* has thick roots (Taylor et al. 2001). The stems, leaves, caudex, roots and 
rhizomes of the four perennials and the grass were dried at 70°C for at least 48 hours 
and weighed.

*Data analysis*

Treatment effects on the survival of the plants were analyzed with a Kaplan-Meier 
Log Rank test per species with bud removal and nutrient enrichment as explaining 
factors in different tests. Prior to statistical analysis the number of rosettes, flowers 
and buds were ln-transformed to improve normality. Dry weights were ln-transformed 
when necessary to increase the homogeneity of the variance of the tested groups. 
Type III ANOVAs were performed on dry weights of plant parts with bud removal, 
fertilization and their interaction as fixed factors and plant pair nested within the 
fertilization treatments as a random factor. Repeated Measures MANOVAs with the
same factors and year as time factor were used for the data on flower and rosette numbers. Type I ANCOVAs (Zar 1996) with vegetative biomass as covariate were performed to test for differential biomass allocation to storage or sexual reproduction. Bud removal and fertilization were the fixed factors in these ANCOVAs. Plant pair was collinear with plant size and thus not included in these models (the plant pairs were designed to differ in plant size and indeed significantly explained variation in harvest size in three species; Table S1).

Decomposing changes in sexual reproduction
Finally, we examined for three species separately (H. radicata was omitted from this analysis due to low survival rates) to what extent the difference in reproductive biomass between the low (C) and high (N) nutrients plots could be explained by changes in plant size, root-shoot ratio or relative somatic costs alone, or together. To assess the explanatory power of plant size, we used the factor by which the mean vegetative biomass of the surviving plants increased in the N compared to the C plots, to predict a mean reproductive biomass in the high nutrient plots, assuming that the allocation patterns in N plots are exactly the same as in the low nutrient plots. Next, we assumed that vegetative biomass and the relative somatic costs in the N plots were the same as in C plots, and examined the change in root-shoot ratio only. A new prediction of reproductive biomass was calculated from the change in root-shoot ratio, based on the assumption that reproductive biomass increases linearly with shoot biomass. Third, we kept the vegetative biomass and the root-shoot ratio as in the C plots and examined the change in the relative somatic costs (Tuomi et al. 1983; Obeso 2002). For a given nutrient treatment $i$, the relative somatic costs of reproduction $\gamma^i$ (i.e. the reduction in vegetative biomass per unit of sexual reproductive biomass removed) was calculated as

$$\gamma^i = \frac{W_{\text{veg}}^{iB} - W_{\text{veg}}^i}{W_{\text{repr}}^i - W_{\text{repr}}^{iB}}$$

in which the increase in mean vegetative biomass ($W_{\text{veg}}$) when flower buds were removed (B), is divided by the concurrent decrease in reproductive biomass ($W_{\text{repr}}$). Reproductive biomass was then predicted to change based on the change in $\gamma$ between the N and C plants and on the assumption of constant costs of all reproductive biomass together. Finally, we combined these three explanations by multiplying their separate effects on reproductive biomass to see if these mechanisms can account for the observed changes in seed production.

Results
Costs of sexual reproduction
Both total biomass (Cirsium dissectum and Centaurea jacea; Fig. 1) and rosette formation (Succisa pratensis and C. jacea; Fig. 2) were significantly enhanced by continuous bud removal, thus showing costs of sexual reproduction for either or both plant growth and clonal propagation in the three longer-lived or clonal species. Similar trends in Hypochaeris radicata were not significant in these whole-year analyses (Fig 2), but significant costs of reproduction were found in the first five months of the experiment (Hartemink et al. 2004). In S. pratensis bud removal caused a large increase in rosette number (10 vs. 4.9) in the final year of the experiment (Fig. 2), whereas rosette number was constant in time in the untreated plants. Apart from
size increases, bud removal also resulted in a small increase in the proportion of the
biomass allocated to the storage organs in *S. pratensis* and *C. jacea*, but not in the
other two species with shorter-lived rosettes (Fig. 3). Bud removal had no effect on
plant survival in all species (Fig. S2). Beside these switches to other life history
functions, bud removal also resulted in efforts to compensate for the lost flower buds.
The number of flower heads and buds of flower heads increased strongly when buds
of flower heads were continuously removed in all species except *C. dissectum* (Fig.
S3).

**Effects of nutrient enrichment and increased competition**

The total biomass of *Molinia caerulea* tripled on average in response to nutrient
addition (91 vs. 279 g, n=320, F=1.47·10^4, p<0.001). Survival in *C. dissectum* and *H.
radicata* was reduced in the enriched plots (Log Rank = 10.50, p=0.001 and Log Rank
= 25.58, p<0.001 respectively; Fig. S2). At harvest survival rates in the high nutrient
group were lowered to 33% in *C. dissectum* and to 13% in *H. radicata* (90% and 65%
respectively for the low nutrient group). Two *C. jacea* plants died and all *S. pratensis*
plants survived. Plants of only the latter two species were able to build up
significantly more biomass (Fig. 1) and rosettes (Fig. 2) when nutrients were given.

Not all individuals in *S. pratensis* were able to increase in size to prevent being
dominated by the grasses, resulting in high plant size variation at the high nutrient
treatment. The effects of nutrient enrichment and bud removal on total biomass were
additive.

When analyzing plant biomass at harvest and the biomass of the flower heads
and seeds produced in the third year of the experiment, both sexual reproductive and
storage biomass were highly significantly correlated with vegetative biomass in all
species (Figs. 3 and 4). Allocation to storage organs did not decrease when
reproductive allocation increased, and increased even slightly in *C. jacea* (Fig. 3).

**Decomposing the increase in sexual reproduction**

The increase in vegetative biomass alone could explain 46% of the observed increase
in mean reproductive biomass in *C. jacea*, but only 21% in *S. pratensis* (Fig.5). In *C.
dissectum*, this percentage was even −11% because the plants were on average smaller
under high than under low nutrient conditions. The root-shoot ratio was significantly
lower in the high nutrients treatment in all species (Table 1). Since shoot biomass
(leaves and stems together) and reproductive biomass were significantly correlated
(Pearson’s coefficient: 0.814), a lowered root-shoot ratio may have increased
reproductive biomass. However, only in *C. dissectum* this effect was considerable
(30% of the observed increase in reproductive biomass; Fig.5).

The relative somatic costs were lower in high nutrient than in the low nutrient
treatments in *C. dissectum* (7.1 vs 2.2 gram vegetative biomass per gram reproductive
biomass) and in *S. pratensis* (4.4 vs 2.1), but there was no difference in *C. jacea* (4.9
vs 4.8). These reductions in relative somatic costs could potentially explain a large
part of the observed increase in reproductive biomass of *S. pratensis* (69%) and *C.*
dissectum (123%) in the high nutrient plots (Fig. 5). Combined the three mechanisms can account for an average 119% of observed increase in mean reproductive biomass.

Discussion

We successfully mimicked biomass accumulation during succession in grasslands with high nutrient influxes by adding nutrients to grassland perennials that were grown in between tussocks of Molinia caerulea. As expected this tall grass increased in biomass after nutrient enrichment, resulting in high mortality rates in the short-lived Hypochaeris radicata and Cirsium dissectum. These two species have a relatively high turnover of leaf biomass, which is disadvantageous when competing with a grass species that accumulates biomass like M. caerulea (Berendse et al. 1987; de Kroon and Bobbink 1997; van der Krift and Berendse 2002). Mortality rates in C. dissectum were lower than in H. radicata, because C. dissectum forms rhizomes and could thus escape from the increasingly dense tussocks. In the other two species, Centaurea jacea and Succisa pratensis, larger plants were able to grow larger and to secure their place in the vegetation. Only these large plants were able to compete with M. caerulea and could benefit from the added nutrients themselves. This is in agreement with Swiss field observations: with increasing site productivity S. pratensis density decreased, but plant size and seed production increased (Billeter et al. 2003).

Costs of sexual reproduction after three years of bud removal

Sexual reproduction has demographic costs in the long run in long-lived perennials, as is exemplified by our results of three years of continuous flower bud removal: inhibition of flower and seed production increased total biomass or rosette number. In S. pratensis bud removal not only caused increases in total biomass but also caused meristemic responses: the number of rosettes increased relatively more than total biomass, probably due to a release of apical dominance of flowering over rosette formation. The method of flower bud removal also induced compensation responses by activation and production of new flower buds (Hartemink et al. 2004). In spite of this additional investment in new flower buds, the method succeeded in revealing costs of sexual reproduction reminiscent of those seen in other studies (Avila-Sakar et al. 2001; Ehrén and van Groenendael 2001; Hartemink et al. 2004).

Both biomass and meristemic responses to flowering inhibition eventually resulted in larger plants, which have higher survival probabilities in natural populations of these species (Jongejans and de Kroon 2005). These demographic trade-offs between sexual reproduction and vegetative growth and survival indicate that it is indeed meaningful to test the hypothesized responses to successional replacement in these perennial herbs by studying shifts in sexual reproductive allocation in relation to investments in other life history functions.

Seed production increased in response to mimicked succession

Our experiment with mimicked succession revealed increased seed production per plant through different processes: by increases in plant size or by increases in allocation to sexual reproduction. The importance of these processes varied strongly between the species. Only for C. jacea, the increase in plant size in high nutrient plots emerged as the most important factor explaining the increase in sexual reproduction. By contrast, in S. pratensis, the 2-3 fold increase in sexual reproduction in nutrient enriched plots was only partly due to a concomitant increase in plant size, although sexual reproduction was highly size-dependent. In this species, a reduction in the relative somatic costs of reproduction decreased considerably in the high nutrient
plots making the largest contribution to the increase in sexual reproduction (Fig. 5). Lower costs of making seeds can especially be expected when increased nutrient availability relaxes the nitrogen limitation of seed production (Loehle 1987; Reekie 1991).

Also in C. dissectum a reduction in the relative somatic costs of reproduction was the major factor contributing to elevated seed production under nutrient enrichment. In this species a lower root-shoot ratio enhanced sexual reproduction too. The lower root-shoot ratios under nutrient enriched conditions may have increased seed production because allocation to all above-ground tissues increased at the expense of allocation to roots, in an attempt to optimize resource acquisition when the plots were fertilized and light rather than nutrients were limiting (Poorter and Nagel 2000). The strong effect of decreased root-shoot ratio in C. dissectum suggests that in this rhizomatous species the shift toward above-ground competition causes more rosettes to flower, which is the only way to form more flower heads in this species (Jongejans et al. 2005).

Sexual reproductive allocation is more flexible than storage allocation, which was found to have a more tight relationship with vegetative biomass. This shows a strong developmental link and less opportunity for flexible storage allocation than for reproductive allocation.

Implications
The results of our three-year experiment show that in order to produce more seeds that may establish in more favorable patches, a plant first has to survive, and that it can only do so by increasing its size to avoid shading (Huber and Wiggerman 1997). Increased sexual reproductive allocation and increased vegetative growth therefore do not exclude each other. Van Zandt et al. (2003) showed that the clonal plant Iris hexagona responds similarly to another type of stress, salinity. Thus empirical evidence is emerging that perennial and clonal species can adjust their life history strategy to adverse growing conditions, confirming model predictions (Sakai 1995; Saikkonen et al. 1998; Gardner and Mangel 1999; Olejniczak 2003).

The responses as revealed in this study have implications for metapopulation dynamics, in which both persistence (patch occupancy) and sexual reproduction (production of diaspores for colonization of empty patches) are key parameters (Eriksson 1996; Soons et al. 2006). Our results suggest that, due to size-dependent costs of seed production, both increased local persistence and enhanced colonization ability through elevated seed production can be combined in a single plant. Such important demographic changes, however, have rarely been incorporated in metapopulation models that focus on succession (e.g. Johnson 2000; Ellner and Fussmann 2003; but see Ronce et al. 2005). Especially for a Red List-species as C. dissectum that only survives in a small number of remnant populations (Soons et al. 2006), seed production level can be a crucial limitation for colonization (Jongejans et al. 2005). When succession advances due to nitrogen deposition or agricultural run-offs, an increase of seed production may be the last sign of life before a population becomes a senile one in which seedlings no longer can establish.

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Literature cited


Lucassen ECHET, Bobbink R, Smolders AJP, van der Ven PJM, Lamers LPM, Roelofs JGM (2003) Interactive effects of low pH and high ammonium levels responsible for the decline of *Cirsium dissectum* (L.) Hill. Plant Ecol 165:45-52


Table 1
Above: ANCOVAs per species (*Hypochaeris radicata*, *Cirsium dissectum*, *Succisa pratensis* and *Centaurea jacea*) on the dry weight (ln g) of the storage organs (caudex) at harvest with bud removal and nutrient addition as fixed factors and vegetative dry weight (roots, leaves and stems) as covariate. The dry weights (ln g) of the flowers in 2002 of the untreated (no bud removal) plants were analyzed the same way. df = degrees of freedom; F = ANCOVA statistic; (*) = p<0.10; * = p<0.05; ** = p<0.01 and *** = p<0.001

Below: ANOVA’s on ln-transformed root-shoot ratios, in which shoots consist of leaves and stems. Only the root-shoot ratios were analyzed of plants of which no buds were removed.

<table>
<thead>
<tr>
<th>Dry Weight</th>
<th>Effect of</th>
<th>H. radicata</th>
<th>C. dissectum</th>
<th>S. pratensis</th>
<th>C. jacea</th>
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<tr>
<td>Storage organs</td>
<td>df F</td>
<td>df F</td>
<td>df F</td>
<td>df F</td>
<td>df F</td>
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<tr>
<td>Intercept</td>
<td>1 27.9 ***</td>
<td>1 409.2 ***</td>
<td>1 1617.2 ***</td>
<td>1 10780.7 ***</td>
<td></td>
</tr>
<tr>
<td>Vegetative Weight (Veg)</td>
<td>1 77.5 ***</td>
<td>1 654.1 ***</td>
<td>1 318.5 ***</td>
<td>1 433.8 ***</td>
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<tr>
<td>Bud removal (Bud)</td>
<td>1 0.2</td>
<td>1 0.6</td>
<td>1 10.6 **</td>
<td>1 4.3 *</td>
<td></td>
</tr>
<tr>
<td>Nutrients (Nutr)</td>
<td>1 0.1</td>
<td>1 0.2</td>
<td>1 3.0 (*)</td>
<td>1 13.9 ***</td>
<td></td>
</tr>
<tr>
<td>Bud * Veg</td>
<td>1 1.4</td>
<td>1 1.9</td>
<td>1 0.1</td>
<td>1 0.5</td>
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<tr>
<td>Bud * Nutr</td>
<td>1 0.1</td>
<td>1 0.1</td>
<td>1 1.3</td>
<td>1 3.7 (*)</td>
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<tr>
<td>Nutr * Veg</td>
<td>1 3.6 (*)</td>
<td>1 1.6</td>
<td>1 1.5</td>
<td>1 2.2</td>
<td></td>
</tr>
<tr>
<td>Bud * Nutr * Veg</td>
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<td>1 2.2</td>
<td>1 0.2</td>
<td>1 0.0</td>
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<td>23 MS=0.31</td>
<td>51 MS=0.10</td>
<td>72 MS=0.13</td>
<td>70 MS=0.082</td>
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<th>Effect of</th>
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<th>C. dissectum</th>
<th>S. pratensis</th>
<th>C. jacea</th>
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<td>Intercept</td>
<td>1 168.8 ***</td>
<td>1 4.6 *</td>
<td>1 958.9 ***</td>
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<td>Vegetative Weight (Veg)</td>
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<td>1 6.2 *</td>
<td>1 49.5 ***</td>
<td>1 0.2</td>
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<tr>
<td>Nutr * Veg</td>
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<td>1 0.6</td>
<td>1 7.0 *</td>
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<tr>
<td>Error</td>
<td>11 MS=0.095</td>
<td>25 MS=0.41</td>
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<th>C. dissectum</th>
<th>S. pratensis</th>
<th>C. jacea</th>
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<td>2 9.9 **</td>
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<td>2 56.0 ***</td>
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Biomass allocation and succession

Figure 1.
Dry weight (g) at harvest divided into sexual reproductive tissue (flower buds, flower heads and seeds), vegetative plant parts (leaves, stems and roots), storage organs (caudex), and rhizomes (C. dissectum only). Downward error bars denote standard errors of the mean weight of plant parts; the upward error bar denotes the standard error of the mean total weight. C = control; B = bud removal; N = nutrients added; NB = bud removal and nutrient addition. Sample sizes at harvest are given below the bars. Significant effects of bud removal (Bud) and nutrient addition (Nutr) are indicated for each species: ns = not significant; (*) = p<0.10; * = p<0.05 and *** = p<0.001. The interactions were not significant (see Table S1 for the complete statistics of the ANOVAs).
**Figure 2.** Development of the number of rosettes (mean ± 1 s.e.) of surviving plants. The nutrient addition treatment started in June 2001. Flower buds were removed all three years. C = control; B = bud removal; N = nutrients added; NB = bud removal and nutrient addition. Significant bud removal (Bud) effects, nutrient (Nutr) effects and year (Yr)-factor interactions are indicated for each species: ns = not significant; (*) = p<0.10; * = p<0.05; ** = p<0.01 and *** = p<0.001. For the complete statistics of the repeated measures MANOVAs see Table S2. For the sample sizes see Fig. S3.
Figure 3.
Dry weight (ln g) of the storage organs (caudex) plotted for each plant against its vegetative (roots, leaves and stems) dry weight (ln g) at harvest. C = control; B = bud removal; N = nutrients added; NB = bud removal and nutrient addition. Significant effects of bud removal (Bud), nutrient addition (Nutr) and the covariate vegetative dry weight (Veg) are indicated for each species: ns = not significant; (*) = p<0.10; * = p<0.05; ** = p<0.01 and *** = p<0.001. The interactions were not significant at the α = 0.05 level. For the complete statistics of the ANCOVAs see Table 1.
Dry weight (\(\ln g\)) of the flowers (flower heads and seeds) plotted per plant against its vegetative (roots, leaves and stems) dry weight (\(\ln g\)) at harvest. C = control; N = nutrients added. Vegetative weight of *C. dissectum* was set back to the moment of flowering by taking the number of rosettes during flowering and multiplying it with the average rosette weight at harvest. Significant effects of nutrient addition (Nutr), the covariate vegetative dry weight (Veg) and their interactions are indicated for each species: ns = not significant; * = \(p<0.05\) and *** = \(p<0.001\). For the complete statistics of the ANCOVAs see Table 1.
Figure 5.

Observed reproductive biomass in the control (C) and nutrient addition (N) group, and four potential explanations for the increase in reproductive biomass from C to N: 1) through the observed increase in vegetative biomass, 2) through the observed decrease in vegetative root-shoot ratio, 3) through the observed decrease in relative somatic costs, and 4) all three previous explanations together. For these explanations it is assumed that reproductive biomass increased linearly with shoot biomass and with total vegetative biomass, and that reproductive biomass increased inversely with a decrease in relative somatic costs. The observed means and standard errors (bars) are rescaled within each species by dividing by the mean biomass in the control group (C). Too few plants survived to do these calculations for Hypochaeris radicata.
**Table S1: Statistics of dry weight.**

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ANOVA’s on dry weight, W (g), at harvest of *Hypochaeris radicata*, *Cirsium dissectum*, *Succisa pratensis* and *Centaurea jacea* with bud removal, nutrient addition and pair of plants as explaining factors. Data were ln-transformed when necessary (ln(W)). ‘Flowers’ = flowers, seeds and removed buds; ‘Vegetative’ = stems, leaves and roots; ‘Storage’ = caudex. df = degrees of freedom, F = ANOVA statistic; (x) = p<0.10; * = p<0.05; ** = p<0.01 and *** = p<0.001
Table S2: Statistics of rosette and flower numbers.

Repeated measures MANOVAs on rosette numbers at harvest and the numbers of flowers or removed buds in the last of the three consecutive years of the allocation experiment with *Hypochaeris radicata*, *Cirsium dissectum*, *Succisa pratensis* and *Centaurea jacea*. Data were transformed (Ln(number + 0.1)) prior to analysis. df = degrees of freedom; F = Wilk’s Lambda statistic; (*) = p<0.10; * = p<0.05; ** = p<0.01 and *** = p<0.001

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Figure S1: Schematic design of the experiment.

The sub block of eight plants represented here either received nutrients, or stayed unfertilized. Each of the twenty blocks contained two adjacent sub blocks with contrasting nutrient treatment. Within a sub block the eight combinations of species and bud removal were randomly distributed over the positions. The sub blocks were fenced with 25 cm deep lawn edging to prevent root growth or leakage of nutrients between nutrient treatments. Ten cm deep lawn edging (50 cm diameter) kept the rhizomatous *Cirsium dissectum* from growing into other plots. Around each target plant six cuttings of *Molinia caerulea* were planted in a hexagon at 10 cm intervals.

*Hr* = Hypochaeris radicata; *Cd* = Cirsium dissectum; *Sp* = Succisa pratensis; *Cj* = Centaurea jacea; *T* = target plant; *M* = *M. caerulea*; *B* = flower bud removal.
Figure S2: Survival percentages over time.

Percentage survival of the plants during the allocation experiment for each combination of the species and the treatments. A letter on the x-axis stands for the 15th day of the month. C = control, N = nutrients added, B = bud removal, NB = bud removal and nutrient addition. There were 20 replicates.
Figure S3: Number of flowers and removed buds.

Development of the number of flowers and removed buds (mean ± standard error) of surviving plants. The nutrient addition treatment started in June 2001. Flower buds were removed all three years. C = control; B = bud removal; N = nutrients added; NB = bud removal and nutrient addition. Significant bud removal (Bud) effects, nutrient (Nutr) effects and year (Yr)-factor interactions are indicated for each species. Cirsium dissectum did not compensate for lost flower buds like the other species, which is probably because C. dissectum has preformed flower buds and a short flowering season. ns = not significant; (*) = p<0.10; * = p<0.05; ** = p<0.01 and *** = p<0.001.

For the complete statistics of the repeated measures MANOVAs see Table S2. The sample sizes were (for all bars from left to right): 20, 20, 20, 19, 18, 18, 12, 12, 13, 2, 3 in H. radicata; 20, 20, 20, 20, 20, 19, 19, 19, 20, 19, 17, 10, 13 in C. dissectum, all 20 in S. pratensis, and 20, 20, 20, 20, 20, 20, 20, 20, 20, 19, 20, 20, 19 in C. jacea.