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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

1 production and dispersal, or by increasing local persistence by adjusting its life history
2 to the changing habitat (Ronce et al. 2005). Since microsites for seedling
3 establishment are often limiting in late-successional grasslands (Kupferschmid et al.
4 2000), plants can extend their local persistence by increasing their size through
5 vegetative and clonal growth to increase competitiveness and to reduce individual
6 mortality rates. Alternatively, plants may enhance flowering and fruiting, and hence
7 increase the chance to escape to new habitats, which may only be possible at the cost
8 of reduced growth and survival (Abrahamson 1980). Whether and how individual
9 plants are able to alter their life history as succession proceeds is yet unclear. Here we
10 study these life history responses experimentally by subjecting four perennial
11 grassland species to simulated successional change.

12 Underlying these hypotheses on adaptive biomass allocation is a trade-off
13 between seed production and vegetative reproduction. Costs of sexual reproduction,
14 i.e. any reduction in fitness parameters like survival, growth, plant size or future
15 reproduction due to biomass investment in sexual reproduction, are a crucial element
16 explaining these alternative hypotheses because in the absence of costs plants could
17 change different life history functions independently. Recent studies have shown that
18 trade-offs can be masked by the size-dependent relationship between plant size and
19 investment into e.g. sexual reproduction (Ågren and Willson 1994; Reznick et al.
20 2000; Ehrlén and van Groenendael 2001). But when plant size is accounted for, trade-
21 offs between life history functions can be found (Méndez and Obeso 1993; Primack
22 and Stacy 1998; Obeso 2002; Hartemink et al. 2004). Therefore we tested for costs of
23 sexual reproduction in this study. We use the method of flower bud removal (Obeso
24 2002) to investigate if plants switch to increased size, storage or vegetative offspring
25 number when flowering and seed set are inhibited.

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

41 **Material and methods**

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

1 because we determined only the costs of the production flowers and seeds (see
2 treatments).

3 *Study species*

4 *Hypochaeris radicata* L. (Asteraceae) is a relatively short-lived perennial. Its leafless
5 flowering stalks and new rosettes are formed clonally by branching of the taproot (de
6 Kroon et al. 1987; Jongejans and de Kroon 2005). Flowering starts in June and
7 continues until autumn.
8

9 *Cirsium dissectum* (L.) Hill (Asteraceae) is a rhizome-forming clonal plant,
10 with monocarpic rosettes. Normally one flower head is formed in June (Jongejans et
11 al. 2005). In the Netherlands *C. dissectum* is a rare and endangered species (Red List
12 2; van der Meijden 1996) due to the decline of its habitat, nutrient-poor moist
13 grasslands (Lucassen et al. 2003).

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

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

27 *Molinia caerulea* (L.) Moench (Poaceae) is a tussock forming, tall grass,
28 which occurs in nutrient-poor grasslands and grass heaths. *Molinia caerulea* starts to
29 dominate when nutrient deposition is high (Berendse and Aerts 1984; Aerts et al.
30 1990), especially when fields are abandoned.

31 *Plant material*

32 Seeds of the four target species were collected in 1998 in the nature reserve
33 'Bennekomse Meent', a nutrient-poor grassland near Wageningen in the Netherlands
34 (52°01'N, 5°36'E; van der Hoek et al. 2004). Cuttings of *M. caerulea* were collected at
35 the same locality. Plants of *C. jacea*, *S. pratensis* and *C. dissectum* were grown from
36 seed in a greenhouse one year before the start of the experiment. In May 2000 newly
37 formed rosettes of these plants were carefully broken off. For *H. radicata* two-
38 months-old seedlings were used. In order to allow for direct comparisons between the
39 bud removal treatment (see below) and the undisturbed plants, all cuttings and
40 seedlings were grouped in pairs of similar initial size, and of the same genetic
41 identity, or, in case of *H. radicata*, grown from seeds of the same mother plant. One
42 plant of each pair was assigned to the bud removal treatment, the other to the
43 untreated group. Each pair of plants was either assigned to the nutrient addition
44 treatment or to the low nutrients treatment. The size-dependency of allocation in an
45 allometric framework was taken into account by starting the experiment with a range
46 of plant sizes rather than selecting for equally sized plants. The cuttings and seedlings
47 were transplanted into an experimental garden of Wageningen University.

48
49 The 320 plants were randomly placed in a randomized block design: four
50 species * two bud removal treatments * two resource treatments * 20 replicates (Fig.

1 S1). The interval between the target plants was 50 cm. Around each target plant six
2 *M. caerulea* clumps of four shoots each were placed in a hexagon with sides of 10 cm.
3 Lawn edging was placed 10 cm deep in a circle of 50 cm in diameter around the *C.*
4 *dissectum* plants to prevent this clonally spreading species from growing through the
5 whole garden. Measurements on 20 additional plots indicated that these circles of
6 lawn edging did not affect the biomass increments of *M. caerulea* (data not shown).

7 *Treatments*

8 Nutrient enrichment was applied to half of the plants in the second and third year,
9 allowing the plants to establish under the same conditions in the first year of the
10 experiment. Nutrient solution was applied to a circular area of 50 cm diameter around
11 the target plants. The Hoagland's stock solution contained KNO_3 , $\text{Ca}(\text{NO}_3)_2$, MgSO_4 ,
12 and $\text{NH}_4\text{H}_2\text{PO}_4$ (Gamborg and Wetter 1975). The solution was applied in three
13 portions within two months at the beginning of the growing season and was
14 equivalent to $120 \text{ kg N ha}^{-1} \text{ yr}^{-1}$, which is about three times the atmospheric deposition
15 in Dutch agricultural landscapes (Bobbink et al. 1998; van Oene et al. 1999). Van der
16 Hoek et al. (2004) found significant shifts in the vegetation composition in the field
17 when they applied $200 \text{ kg N ha}^{-1} \text{ yr}^{-1}$. In each of the 20 blocks all plants that were
18 assigned to the high nutrient treatment were placed together and surrounded with 25
19 cm-deep lawn edging to prevent nutrient leakage to the plants of the low nutrients
20 group (Fig. S1). The unfertilized plants of each block were also grouped and enclosed
21 by the same lawn edging.

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

25 *Measurements*

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

40 *Data analysis*

41
42 Treatment effects on the survival of the plants were analyzed with a Kaplan-Meier
43 Log Rank test per species with bud removal and nutrient enrichment as explaining
44 factors in different tests. Prior to statistical analysis the number of rosettes, flowers
45 and buds were ln-transformed to improve normality. Dry weights were ln-transformed
46 when necessary to increase the homogeneity of the variance of the tested groups.
47 Type III ANOVAs were performed on dry weights of plant parts with bud removal,
48 fertilization and their interaction as fixed factors and plant pair nested within the
49 fertilization treatments as a random factor. Repeated Measures MANOVAs with the
50

1 same factors and year as time factor were used for the data on flower and rosette
 2 numbers. Type I ANCOVAs (Zar 1996) with vegetative biomass as covariate were
 3 performed to test for differential biomass allocation to storage or sexual reproduction.
 4 Bud removal and fertilization were the fixed factors in these ANCOVAs. Plant pair
 5 was collinear with plant size and thus not included in these models (the plant pairs
 6 were designed to differ in plant size and indeed significantly explained variation in
 7 harvest size in three species; Table S1).

9 *Decomposing changes in sexual reproduction*

10 Finally, we examined for three species separately (*H. radicata* was omitted from this
 11 analysis due to low survival rates) to what extent the difference in reproductive
 12 biomass between the low (C) and high (N) nutrients plots could be explained by
 13 changes in plant size, root-shoot ratio or relative somatic costs alone, or together. To
 14 assess the explanatory power of plant size, we used the factor by which the mean
 15 vegetative biomass of the surviving plants increased in the N compared to the C plots,
 16 to predict a mean reproductive biomass in the high nutrient plots, assuming that the
 17 allocation patterns in N plots are exactly the same as in the low nutrient plots. Next,
 18 we assumed that vegetative biomass and the relative somatic costs in the N plots were
 19 the same as in C plots, and examined the change in root-shoot ratio only. A new
 20 prediction of reproductive biomass was calculated from the change in root-shoot ratio,
 21 based on the assumption that reproductive biomass increases linearly with shoot
 22 biomass. Third, we kept the vegetative biomass and the root-shoot ratio as in the C
 23 plots and examined the change in the relative somatic costs (Tuomi et al. 1983; Obeso
 24 2002). For a given nutrient treatment i , the relative somatic costs of reproduction γ^i
 25 (i.e. the reduction in vegetative biomass per unit of sexual reproductive biomass
 26 removed) was calculated as

$$27 \quad \gamma^i = \frac{\overline{W_{veg}^{iB}} - \overline{W_{veg}^i}}{\overline{W_{repr}^i} - \overline{W_{repr}^{iB}}} \quad (1)$$

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

36 **Results**

37 *Costs of sexual reproduction*

38 Both total biomass (*Cirsium dissectum* and *Centaurea jacea*; Fig. 1) and rosette
 39 formation (*Succisa pratensis* and *C. jacea*; Fig. 2) were significantly enhanced by
 40 continuous bud removal, thus showing costs of sexual reproduction for either or both
 41 plant growth and clonal propagation in the three longer-lived or clonal species.
 42 Similar trends in *Hypochaeris radicata* were not significant in these whole-year
 43 analyses (Fig 2), but significant costs of reproduction were found in the first five
 44 months of the experiment (Hartemink et al. 2004). In *S. pratensis* bud removal caused
 45 a large increase in rosette number (10 vs. 4.9) in the final year of the experiment (Fig.
 46 2), whereas rosette number was constant in time in the untreated plants. Apart from

1 size increases, bud removal also resulted in a small increase in the proportion of the
2 biomass allocated to the storage organs in *S. pratensis* and *C. jacea*, but not in the
3 other two species with shorter-lived rosettes (Fig. 3). Bud removal had no effect on
4 plant survival in all species (Fig. S2). Beside these switches to other life history
5 functions, bud removal also resulted in efforts to compensate for the lost flower buds.
6 The number of flower heads and buds of flower heads increased strongly when buds
7 of flower heads were continuously removed in all species except *C. dissectum* (Fig.
8 S3).

9 *Effects of nutrient enrichment and increased competition*

10 The total biomass of *Molinia caerulea* tripled on average in response to nutrient
11 addition (91 vs. 279 g, $n=320$, $F=1.47 \cdot 10^4$, $p<0.001$). Survival in *C. dissectum* and *H.*
12 *radicata* was reduced in the enriched plots (Log Rank = 10.50, $p=0.001$ and Log Rank
13 = 25.58, $p<0.001$ respectively; Fig. S2). At harvest survival rates in the high nutrient
14 group were lowered to 33% in *C. dissectum* and to 13% in *H. radicata* (90% and 65%
15 respectively for the low nutrient group). Two *C. jacea* plants died and all *S. pratensis*
16 plants survived. Plants of only the latter two species were able to build up
17 significantly more biomass (Fig. 1) and rosettes (Fig. 2) when nutrients were given.
18 Not all individuals in *S. pratensis* were able to increase in size to prevent being
19 dominated by the grasses, resulting in high plant size variation at the high nutrient
20 treatment. The effects of nutrient enrichment and bud removal on total biomass were
21 additive.
22

23 When analyzing plant biomass at harvest and the biomass of the flower heads
24 and seeds produced in the third year of the experiment, both sexual reproductive and
25 storage biomass were highly significantly correlated with vegetative biomass in all
26 species (Figs. 3 and 4). Allocation to storage organs did not decrease when
27 reproductive allocation increased, and increased even slightly in *C. jacea* (Fig. 3).
28 Nutrient enrichment had significantly positive effects on reproductive allocation in *S.*
29 *pratensis*, *C. dissectum* and *H. radicata* (although the sample sizes in the last species
30 were small). In *C. jacea* the regression lines of the low and high nutrient groups
31 intersected (Fig. 4), indicating that in the enriched group larger plants produced more
32 seeds and smaller plants less seeds than equally-sized untreated plants. In summary,
33 sexual reproduction increased significantly (in all species except *H. radicata*) under
34 simulated successional change through nutrient addition (Table S1).
35

36 *Decomposing the increase in sexual reproduction*

37 The increase in vegetative biomass alone could explain 46% of the observed increase
38 in mean reproductive biomass in *C. jacea*, but only 21% in *S. pratensis* (Fig.5). In *C.*
39 *dissectum*, this percentage was even -11% because the plants were on average smaller
40 under high than under low nutrient conditions. The root-shoot ratio was significantly
41 lower in the high nutrients treatment in all species (Table 1). Since shoot biomass
42 (leaves and stems together) and reproductive biomass were significantly correlated
43 (Pearson's coefficient: 0.814), a lowered root-shoot ratio may have increased
44 reproductive biomass. However, only in *C. dissectum* this effect was considerable
45 (30% of the observed increase in reproductive biomass; Fig.5).

46 The relative somatic costs were lower in high nutrient than in the low nutrient
47 treatments in *C. dissectum* (7.1 vs 2.2 gram vegetative biomass per gram reproductive
48 biomass) and in *S. pratensis* (4.4 vs 2.1), but there was no difference in *C. jacea* (4.9
49 vs 4.8). These reductions in relative somatic costs could potentially explain a large
50 part of the observed increase in reproductive biomass of *S. pratensis* (69%) and *C.*

1 *dissectum* (123%) in the high nutrient plots (Fig. 5). Combined the three mechanisms
2 can account for on average 119% of observed increase in mean reproductive biomass.
3

4 **Discussion**

5 We successfully mimicked biomass accumulation during succession in grasslands
6 with high nutrient influxes by adding nutrients to grassland perennials that were
7 grown in between tussocks of *Molinia caerulea*. As expected this tall grass increased
8 in biomass after nutrient enrichment, resulting in high mortality rates in the short-
9 lived *Hypochaeris radicata* and *Cirsium dissectum*. These two species have a
10 relatively high turnover of leaf biomass, which is disadvantageous when competing
11 with a grass species that accumulates biomass like *M. caerulea* (Berendse et al. 1987;
12 de Kroon and Bobbink 1997; van der Krift and Berendse 2002). Mortality rates in *C.*
13 *dissectum* were lower than in *H. radicata*, because *C. dissectum* forms rhizomes and
14 could thus escape from the increasingly dense tussocks. In the other two species,
15 *Centaurea jacea* and *Succisa pratensis*, larger plants were able to grow larger and to
16 secure their place in the vegetation. Only these large plants were able to compete with
17 *M. caerulea* and could benefit from the added nutrients themselves. This is in
18 agreement with Swiss field observations: with increasing site productivity *S. pratensis*
19 density decreased, but plant size and seed production increased (Billeter et al. 2003).
20

21 *Costs of sexual reproduction after three years of bud removal*

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

33 Both biomass and meristemic responses to flowering inhibition eventually
34 resulted in larger plants, which have higher survival probabilities in natural
35 populations of these species (Jongejans and de Kroon 2005). These demographic
36 trade-offs between sexual reproduction and vegetative growth and survival indicate
37 that it is indeed meaningful to test the hypothesized responses to successional
38 replacement in these perennial herbs by studying shifts in sexual reproductive
39 allocation in relation to investments in other life history functions.
40

41 *Seed production increased in response to mimicked succession*

42 Our experiment with mimicked succession revealed increased seed production per
43 plant through different processes: by increases in plant size or by increases in
44 allocation to sexual reproduction. The importance of these processes varied strongly
45 between the species. Only for *C. jacea*, the increase in plant size in high nutrient plots
46 emerged as the most important factor explaining the increase in sexual reproduction.
47 By contrast, in *S. pratensis*, the 2-3 fold increase in sexual reproduction in nutrient
48 enriched plots was only partly due to a concomitant increase in plant size, although
49 sexual reproduction was highly size-dependent. In this species, a reduction in the
50 relative somatic costs of reproduction decreased considerably in the high nutrient

1 plots making the largest contribution to the increase in sexual reproduction (Fig. 5).
2 Lower costs of making seeds can especially be expected when increased nutrient
3 availability relaxes the nitrogen limitation of seed production (Loehle 1987; Reekie
4 1991).

5 Also in *C. dissectum* a reduction in the relative somatic costs of reproduction
6 was the major factor contributing to elevated seed production under nutrient
7 enrichment. In this species a lower root-shoot ratio enhanced sexual reproduction too.
8 The lower root-shoot ratios under nutrient enriched conditions may have increased
9 seed production because allocation to all above-ground tissues increased at the
10 expense of allocation to roots, in an attempt to optimize resource acquisition when the
11 plots were fertilized and light rather than nutrients were limiting (Poorter and Nagel
12 2000). The strong effect of decreased root-shoot ratio in *C. dissectum* suggests that in
13 this rhizomatous species the shift toward above-ground competition causes more
14 rosettes to flower, which is the only way to form more flower heads in this species
15 (Jongejans et al. 2005).

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

20 21 *Implications*

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

31 The responses as revealed in this study have implications for metapopulation
32 dynamics, in which both persistence (patch occupancy) and sexual reproduction
33 (production of diaspores for colonization of empty patches) are key parameters
34 (Eriksson 1996; Soons et al. 2006). Our results suggest that, due to size-dependent
35 costs of seed production, both increased local persistence and enhanced colonization
36 ability through elevated seed production can be combined in a single plant. Such
37 important demographic changes, however, have rarely been incorporated in
38 metapopulation models that focus on succession (e.g. Johnson 2000; Ellner and
39 Fussmann 2003; but see Ronce et al. 2005). Especially for a Red List-species as *C.*
40 *dissectum* that only survives in a small number of remnant populations (Soons et al.
41 2006), seed production level can be a crucial limitation for colonization (Jongejans et
42 al. 2005). When succession advances due to nitrogen deposition or agricultural run-
43 offs, an increase of seed production may be the last sign of life before a population
44 becomes a senile one in which seedlings no longer can establish.

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1 **Table 1**

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

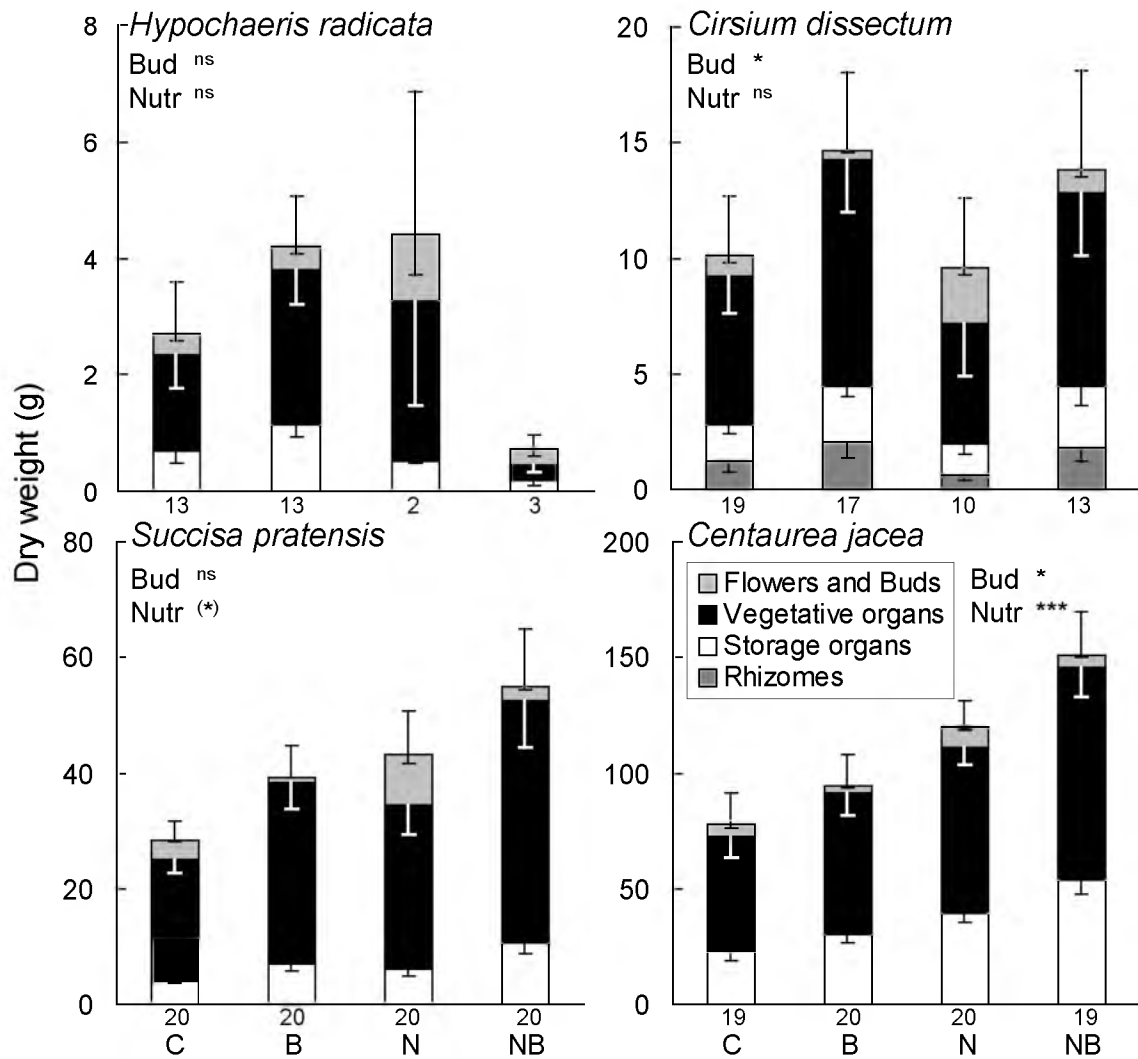
9 Below: ANOVA's on ln-transformed root-shoot ratios, in which shoots consist of
 10 leaves and stems. Only the root-shoot ratios were analyzed of plants of which no buds
 11 were removed.

<i>Dry Weight</i>	Effect of	<i>H. radicata</i>		<i>C. dissectum</i>		<i>S. pratensis</i>		<i>C. jacea</i>	
		df	F	df	F	df	F	df	F
<i>Storage organs</i>									
Intercept		1	27.9 ***	1	409.2 ***	1	1617.2 ***	1	10780.7 ***
Vegetative Weight (Veg)		1	77.5 ***	1	854.1 ***	1	318.5 ***	1	433.8 ***
Bud removal (Bud)		1	0.2	1	0.6	1	10.6 **	1	4.3 *
Nutrients (Nutr)		1	0.1	1	0.2	1	3.0 (*)	1	13.9 ***
Bud * Nutr		1	1.4	1	1.9	1	0.1	1	0.5
Bud * Veg		1	0.1	1	0.1	1	1.3	1	3.7 (*)
Nutr * Veg		1	3.6 (*)	1	1.6	1	1.5	1	2.2
Bud * Nutr * Veg		1	0.1	1	2.2	1	0.2	1	0.0
Error		23	MS=0.31	51	MS=0.10	72	MS=0.13	70	MS=0.082
<i>Flowers</i>									
Intercept		1	166.5 ***	1	4.6 *	1	958.9 ***	1	223.1 ***
Vegetative Weight (Veg)		1	138.8 ***	1	95.9 ***	1	134.0 ***	1	50.1 ***
Nutrients (Nutr)		1	4.9 *	1	6.2 *	1	49.5 ***	1	0.2
Nutr * Veg		1	0.0	1	2.5	1	0.6	1	7.0 *
Error		11	MS=0.095	25	MS=0.41	36	MS=0.096	35	MS=0.44
<i>Ratio</i>									
	Effect of	df	F	df	F	df	F	df	F
<i>Root-Shoot</i>									
Nutrients (Nutr)		2	9.9 **	2	81.5 ***	2	56.0 ***	2	25.4 ***
Error		13	MS=0.40	27	MS=0.20	38	MS=0.22	37	MS=0.18

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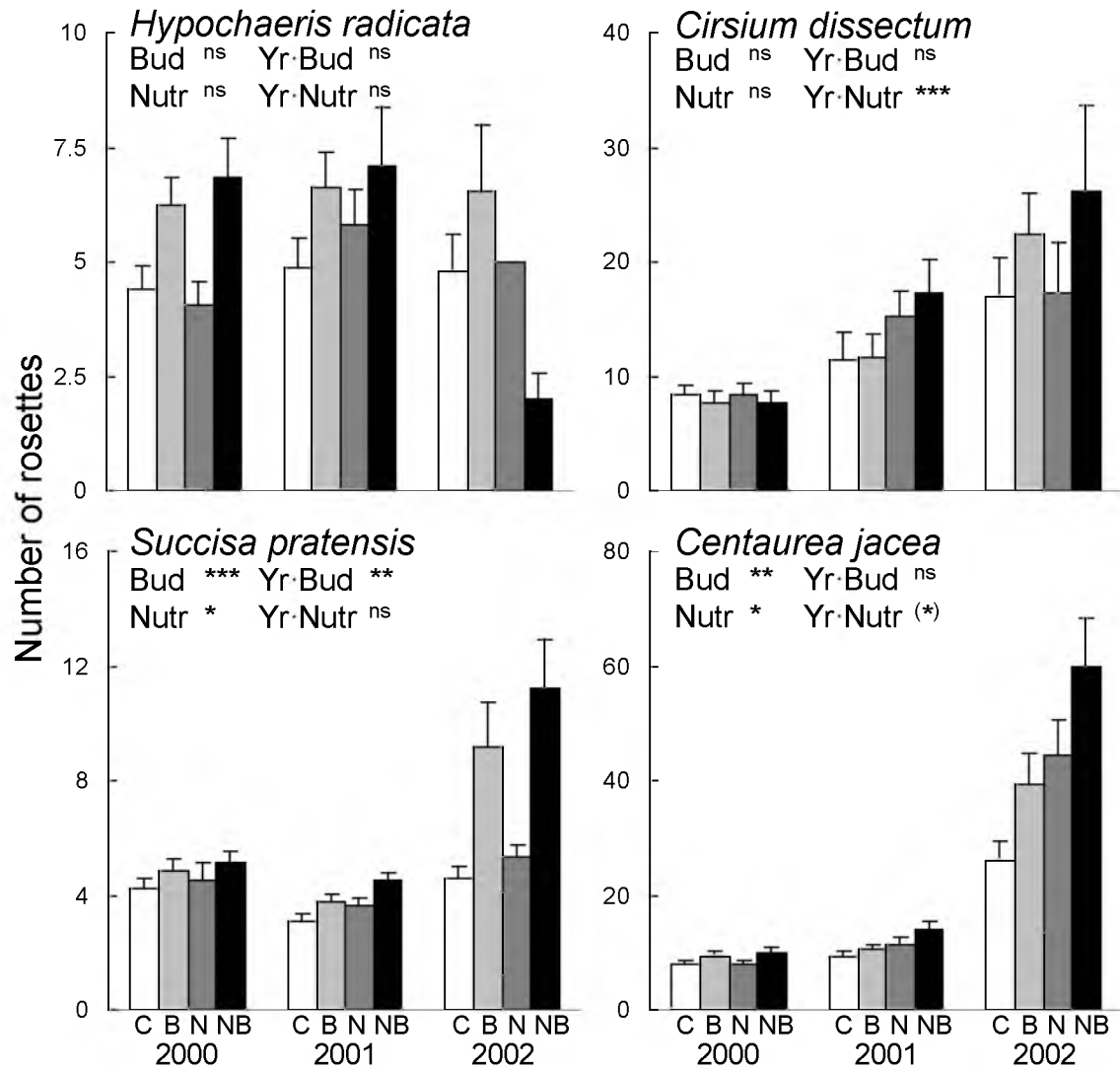
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Figure 1.

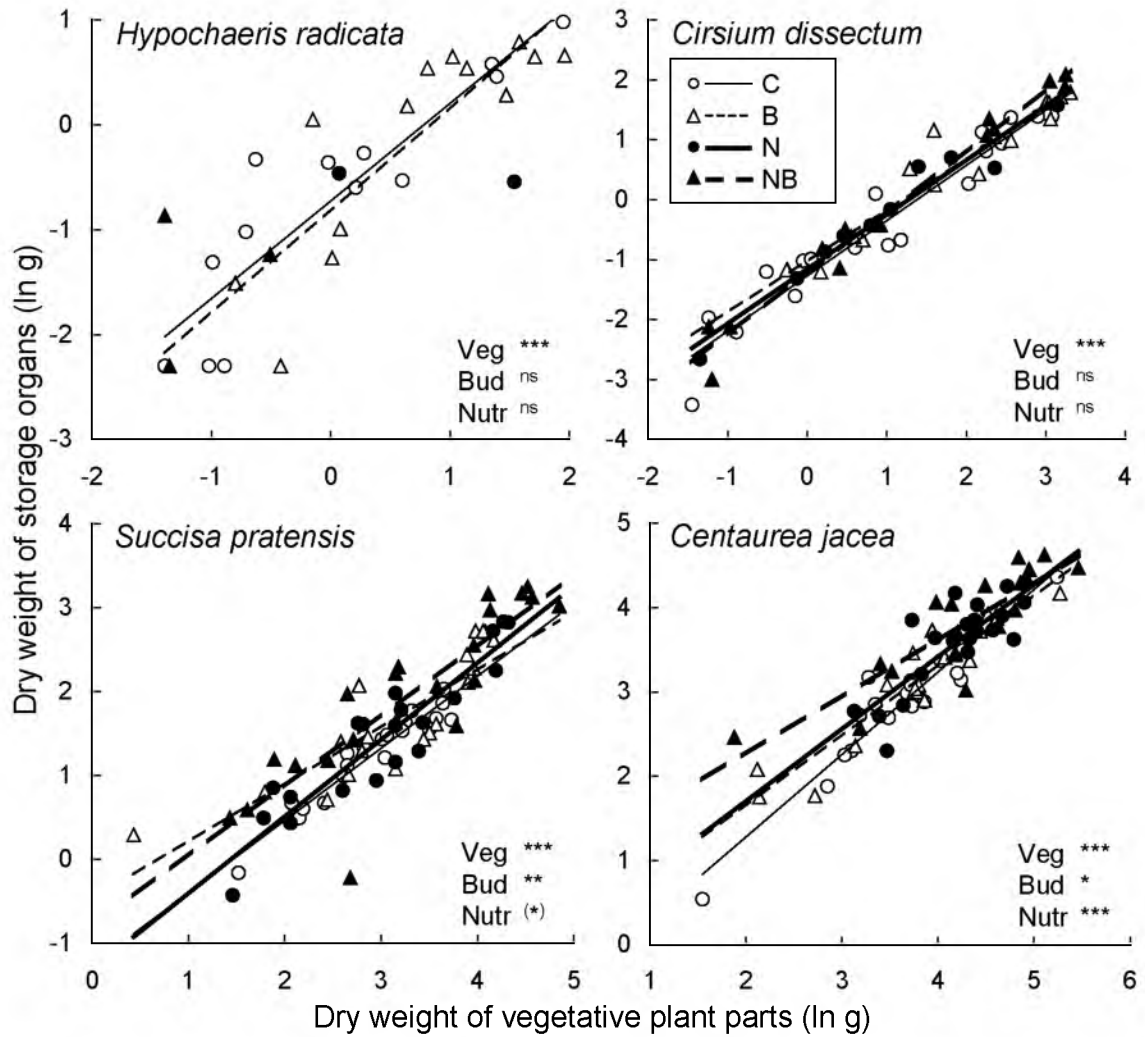
Dry weight (g) at harvest divided into sexual reproductive tissue (flower buds, flower heads and seeds), vegetative plant parts (leaves, stems and roots), storage organs (caudex), and rhizomes (*C. dissectum* only). Downward error bars denote standard errors of the mean weight of plant parts; the upward 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).



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Figure 2.

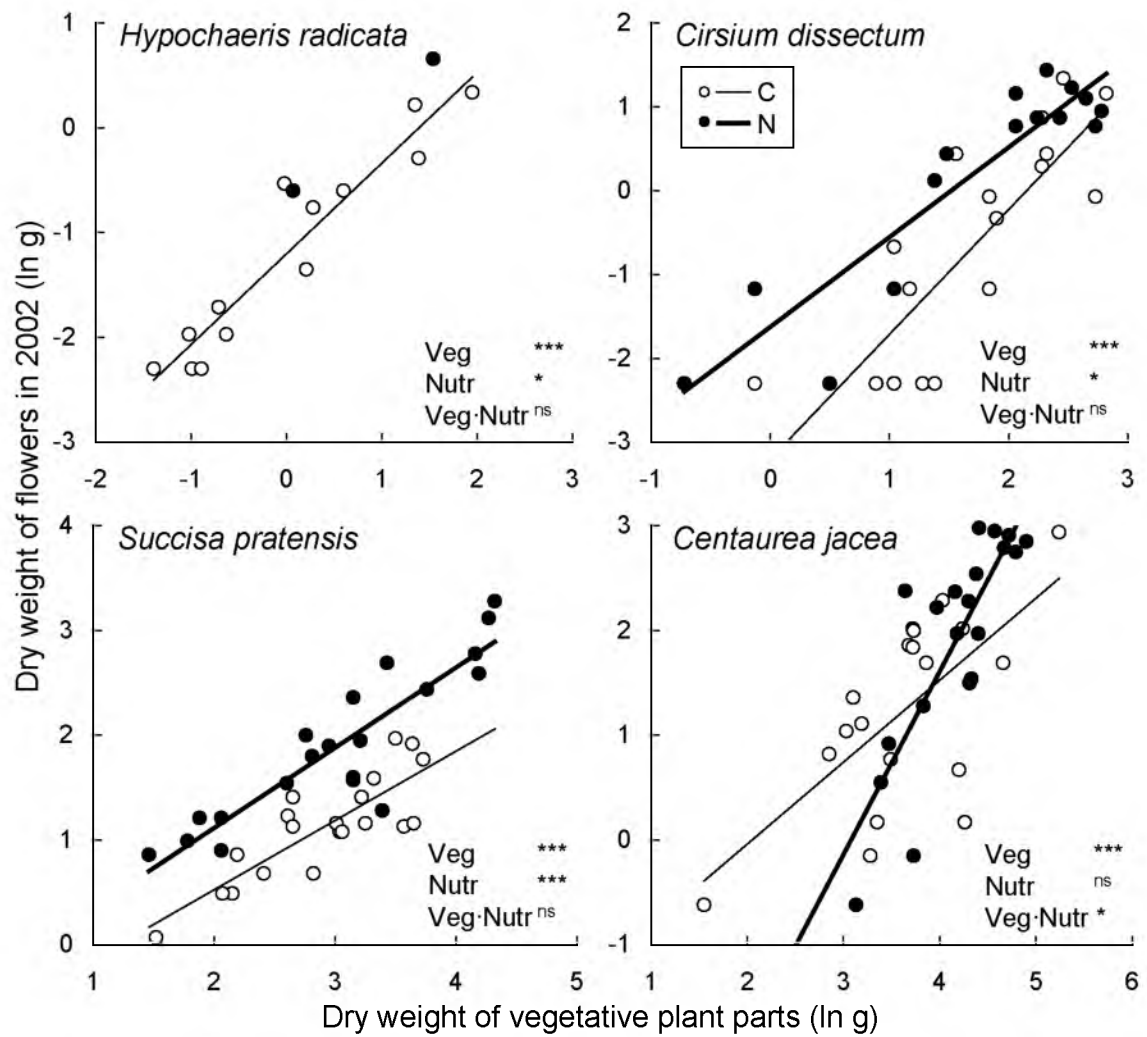
Development of the number of rosettes (mean + 1 s.e.) of surviving plants. The nutrient addition treatment started in June 2001. Flower buds were removed all three years. C = control; B = bud removal; N = nutrients added; NB = bud removal and nutrient addition. Significant bud removal (Bud) effects, 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.



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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 $\alpha = 0.05$ level. For the complete statistics of the ANCOVAs see Table 1.

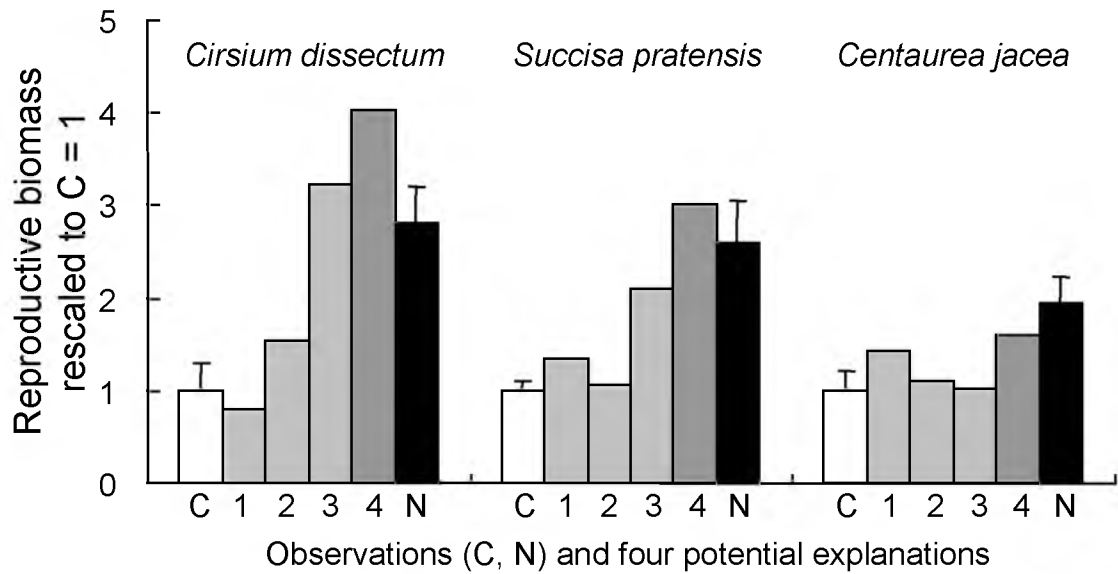


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Figure 4.

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.

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Figure 5.

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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*.

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1 **Table S1: Statistics of dry weight.**

Dry Weight	Effect	<i>H. radicata</i>		<i>C. dissectum</i>		<i>S. pratensis</i>		<i>C. jacea</i>	
		df	F	df	F	df	F	df	F
Total		W		W		ln(W)		W	
	Intercept	1	19.8 **	1	42.6 ***	1	3153.3 ***	1	397.1 ***
	Pair (within Nutr)	20	1.2	33	1.7 (*)	38	3.7 ***	38	2.7 **
	Bud removal (Bud)	1	1.0	1	4.5 *	1	1.8	1	4.7 *
	Nutrients (Nutr)	1	0.6	1	0.8	1	3.3 (*)	1	20.9 ***
	Bud * Nutr	0	-	1	0.9	1	0.1	1	0.1
	Error		7 MS=7.98	22 MS=116.60	38 MS=0.30	36 MS=2257.95			
Flowers		W		W		ln(W)		ln(W)	
	Intercept	1	22.4 **	1	64.4 ***	1	210.6 ***	1	160.0 ***
	Pair (within Nutr)	20	0.9	33	1.0	38	2.6 **	38	2.4 **
	Bud removal (Bud)	1	0.1	1	8.8 **	1	106.8 ***	1	16.1 ***
	Nutrients (Nutr)	1	1.8	1	13.0 **	1	36.5 ***	1	18.3 ***
	Bud * Nutr	0	-	1	1.3	1	0.1	1	0.0
	Error		7 MS=0.22	22 MS=0.86	37 MS=0.30	36 MS=0.63			
Vegetative		W		W		ln(W)		W	
	Intercept	1	17.0 **	1	37.1 **	1	2227.2 ***	1	350.7 ***
	Pair (within Nutr)	20	1.2	33	1.7	38	3.5 ***	38	2.9 **
	Bud removal (Bud)	1	0.9	1	4.8 *	1	3.9 (*)	1	5.2 *
	Nutrients (Nutr)	1	0.7	1	1.7	1	1.0	1	13.4 **
	Bud * Nutr	0	-	1	0.8	1	0.0	1	0.1
	Error		7 MS=3.52	22 MS=51.04	38 MS=0.34	36 MS=983.96			
Storage		W		ln(W)		ln(W)		ln(W)	
	Intercept	1	17.9 **	1	4.0 (*)	1	901.4 ***	1	3995.9 ***
	Pair (within Nutr)	20	1.5	33	1.8 (*)	38	4.3 ***	38	3.3 ***
	Bud removal (Bud)	1	2.3	1	4.6 *	1	19.2 ***	1	7.6 **
	Nutrients (Nutr)	1	3.7 (*)	1	2.7	1	5.0 *	1	37.6 ***
	Bud * Nutr	0	-	1	0.2	1	0.1	1	0.6
	Error		7 MS=0.39	22 MS=1.29	38 MS=0.23	36 MS=0.21			
Rhizomes				ln(W)					
	Intercept			1	12.7 **				
	Pair (within Nutr)			29	1.5				
	Bud removal (Bud)			1	3.6 (*)				
	Nutrients (Nutr)			1	0.1				
	Error			18 MS=2.93					

2

3 ANOVA's on dry weight, W (g), at harvest of *Hypochaeris radicata*, *Cirsium*
4 *dissectum*, *Succisa pratensis* and *Centaurea jacea* with bud removal, nutrient addition
5 and pair of plants as explaining factors. Data were ln-transformed when necessary
6 (ln(W)). 'Flowers' = flowers, seeds and removed buds; 'Vegetative' = stems, leaves
7 and roots; 'Storage' = caudex. df = degrees of freedom; F = ANOVA statistic; (*) =
8 p<0.10; * = p<0.05; ** = p<0.01 and *** = p<0.001

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1 **Table S2: Statistics of rosette and flower numbers.**

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

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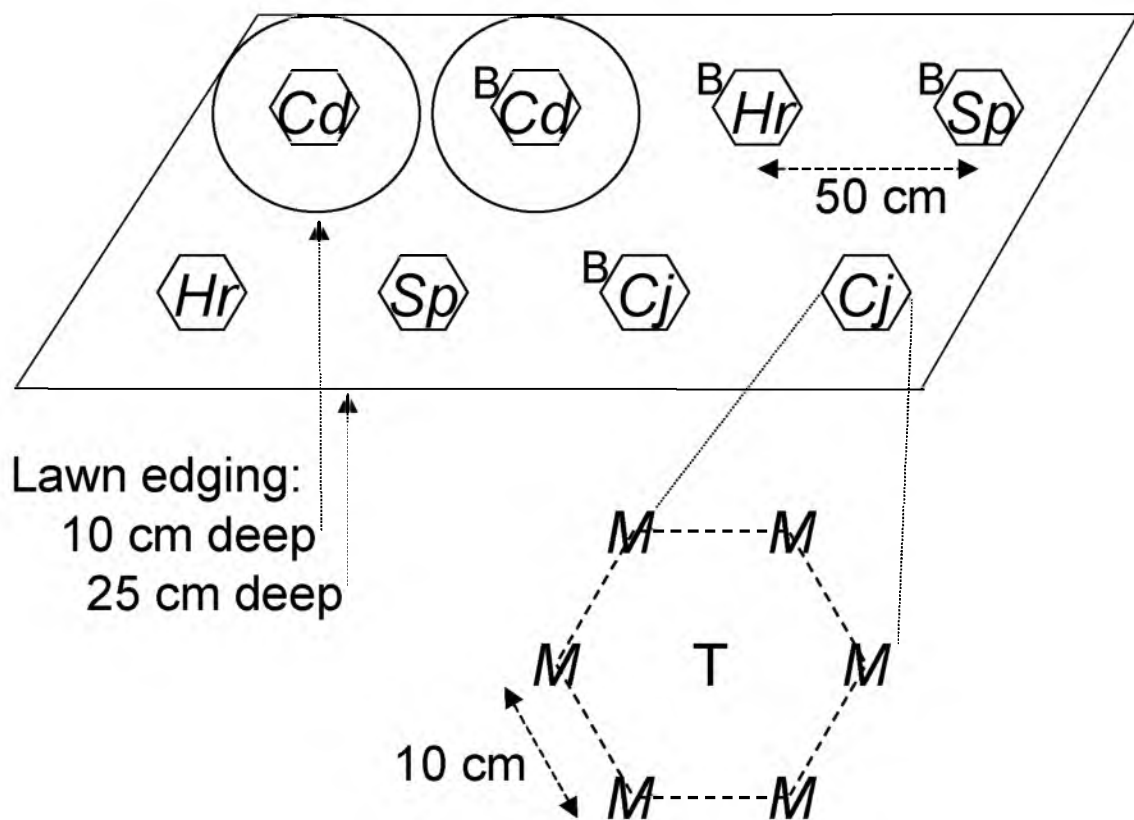
Number of	Effect of	<i>H. radicata</i>		<i>C. dissectum</i>		<i>S. pratensis</i>		<i>C. jacea</i>	
		df	F	df	F	df	F	df	F
Rosettes									
<i>Between subjects</i>									
	Intercept	1	120.1 ***	1	818.5 ***	1	2612.4 ***	1	3645.0 ***
	Pair (within Nutr)	20	1.3	33	2.9 **	38	2.7 **	38	2.9 **
	Bud removal (Bud)	1	0.7	1	0.8	1	31.4 ***	1	8.5 **
	Nutrients (Nutr)	1	0.1	1	0.0	1	4.9 *	1	6.3 *
	Bud * Nutr	0	-	1	0.0	1	0.2	1	0.0
	Error	7	MS=0.87	22	MS=0.87	38	MS=0.21	36	MS=0.42
<i>Within subjects</i>									
	Year (Yr)	2	2.5	2	9.4 **	2	55.6 ***	2	88.3 ***
	Yr * Pair (within Nutr)	40	6.4 **	66	1.9 *	76	1.4 (*)	76	1.4
	Yr * Bud removal (Bud)	2	0.2	2	1.8	2	8.5 **	2	0.4
	Yr * Nutrients (Nutr)	2	1.8	2	12.7 ***	2	1.4	2	3.1 (*)
	Yr * Bud * Nutr	0	-	2	0.3	2	0.2	2	0.4
Flowers or Removed Buds									
<i>Between subjects</i>									
	Intercept	1	420.0 ***	1	3.0 (*)	1	5074.4 ***	1	2407.9 ***
	Pair (within Nutr)	20	2.1	33	2.2 *	38	7.1 ***	38	2.1 *
	Bud removal (Bud)	1	35.5 **	1	0.5	1	85.5 ***	1	40.1 ***
	Nutrients (Nutr)	1	0.2	1	8.5 **	1	11.3 **	1	10.4 **
	Bud * Nutr	0	-	1	1.1	1	0.7	1	0.0
	Error	7	MS=1.61	22	MS=1.38	38	MS=0.55	36	MS=1.25
<i>Within subjects</i>									
	Year (Yr)	2	37.6 ***	2	107.9 ***	2	560.3 ***	2	80.0 ***
	Yr * Pair (within Nutr)	40	2.4 (*)	66	1.2	76	4.1 ***	76	1.3
	Yr * Bud removal (Bud)	2	6.0 *	2	1.2	2	4.7 *	2	0.4
	Yr * Nutrients (Nutr)	2	4.0 (*)	2	3.7 *	2	11.5 ***	2	5.4 **
	Yr * Bud * Nutr	0	-	2	0.2	2	2.1	2	0.8

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1 **Figure S1: Schematic design of the experiment.**

2 The sub block of eight plants represented here either received nutrients, or stayed
 3 unfertilized. Each of the twenty blocks contained two adjacent sub blocks with
 4 contrasting nutrient treatment. Within a sub block the eight combinations of species
 5 and bud removal were randomly distributed over the positions. The sub blocks were
 6 fenced with 25 cm deep lawn edging to prevent root growth or leakage of nutrients
 7 between nutrient treatments. Ten cm deep lawn edging (50 cm diameter) kept the
 8 rhizomatous *Cirsium dissectum* from growing into other plots. Around each target
 9 plant six cuttings of *Molinia caerulea* were planted in a hexagon at 10 cm intervals.
 10 *Hr* = *Hypochaeris radicata*; *Cd* = *Cirsium dissectum*; *Sp* = *Succisa pratensis*; *Cj* =
 11 *Centaurea jacea*; T = target plant; M = *M. caerulea*; B = flower bud removal.

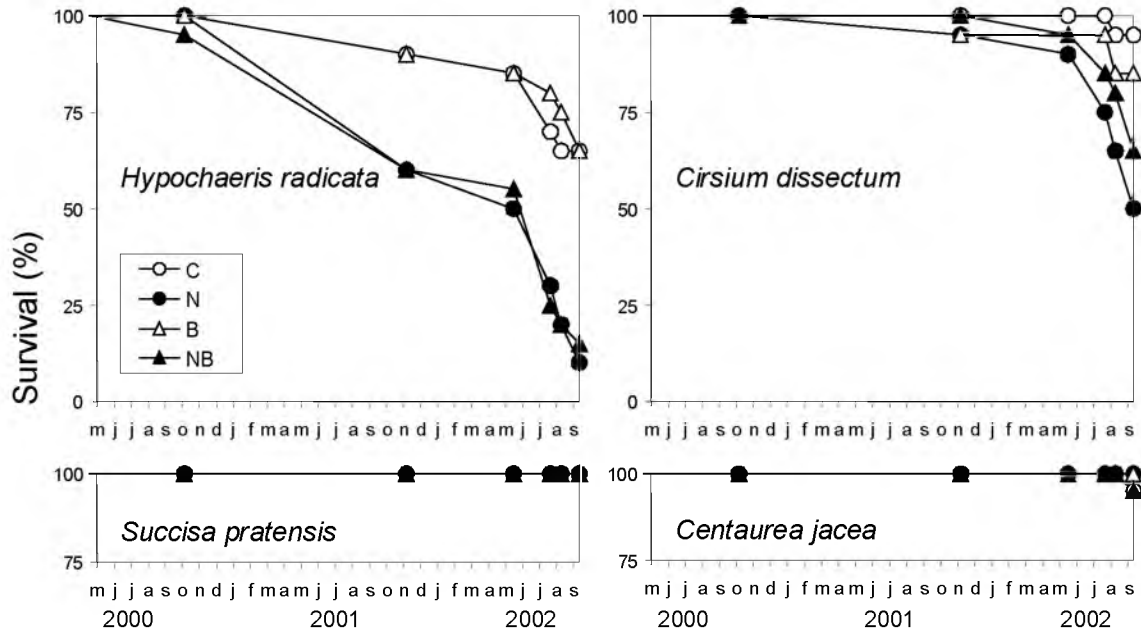
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1 **Figure S2: Survival percentages over time.**

2 Percentage survival of the plants during the allocation experiment for each
 3 combination of the species and the treatments. A letter on the x-axis stands for the 15th
 4 day of the month. C = control; N = nutrients added; B = bud removal; NB = bud
 5 removal and nutrient addition. There were 20 replicates.



6

1 **Figure S3: Number of flowers and removed buds.**

2 Development of the number of flowers and removed buds (mean + standard error) of
 3 surviving plants. The nutrient addition treatment started in June 2001. Flower buds
 4 were removed all three years. C = control; B = bud removal; N = nutrients added; NB
 5 = bud removal and nutrient addition. Significant bud removal (Bud) effects, nutrient
 6 (Nutr) effects and year (Yr)-factor interactions are indicated for each species. *Cirsium*
 7 *dissectum* did not compensate for lost flower buds like the other species, which is
 8 probably because *C. dissectum* has preformed flower buds and a short flowering
 9 season. ns = not significant; (*) = p<0.10; * = p<0.05; ** = p<0.01 and *** = p<0.001.
 10 For the complete statistics of the repeated measures MANOVAs see Table S2. The
 11 sample sizes were (for all bars from left to right): 20, 20, 20, 19, 18, 18, 12, 12, 13,
 12 13, 2, 3 in *H. radicata*; 20, 20, 20, 20, 20, 19, 19, 20, 19, 17, 10, 13 in *C. dissectum*,
 13 all 20 in *S. pratensis*, and 20, 20, 20, 20, 20, 20, 20, 20, 19, 20, 20, 19 in *C. jacea*.

