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Effects of slug herbivory on the seedling establishment of two montane Asteraceae species

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Abstract

Seedling establishment as the life stage transition most sensitive to herbivory might be impossible even if the herbivory losses suffered by adult plants of the same species are tolerable. We tested the hypothesis that herbivory impedes seedling establishment of two montane Asteraceae species on their lower altitudinal distribution border.

In a submontane meadow in the Harz Mountains, Germany, the montane grassland perennials *Arnica montana* and *Centaurea pseudophrygia* (Asteraceae) were sown into artificially created gaps of two different sizes, half of which were protected from slug grazing by molluscicide application in the initial phase of the experiments. The *Arnica* experiments started in spring and late summer of 1999 and in spring of 2000; whereas *Centaurea* was only sown in the spring of 2000. Seedling survival was monitored until the autumn of 2000.

Ten weeks after sowing, mollusc exclusion resulted in significantly higher *Arnica* or *Centaurea* seedling numbers in all experiments; whereas grasses and dicotyledons that emerged from the seedbank were promoted by slug exclosure in only one experiment. Species diversity was not affected by molluscicide application. By the end of the second growing season, the survivorship of *Arnica* sown in the spring of 1999 was reduced to less than 1% of the number of sown seeds in the gaps with natural slug grazing, compared to 7–15% in the gaps with molluscicide treatment. Plant density of most species was higher in the small than in the large gaps, indicating a microclimatic shelter effect of the surrounding vegetation.

The results show that slug herbivory might exert large effects on species composition in grassland; it does strongly influence the seedling establishment of the highly palatable, slow-growing *A. montana*, while the seedlings of *C. pseudophrygia*, although of similar palatability, are more able to tolerate slug feeding because of their higher growth rate.

Keywords: Molluscs; *Arnica montana*; *Centaurea pseudophrygia*; Exclosure; Gap size; Plant–animal interactions

Introduction

Seedling establishment is the crucial point in a species' life cycle. Even populations of long-lived perennial plant species have to regenerate by seed in the long term, and therefore need safe sites for seedling establishment. The

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main causes of seedling death are competition from neighbouring plants and herbivory, or interactions of these two (Fenner, 1987). Plant species which are characterized by a slow growth and a high palatability may therefore face immense problems in their seedling establishment. In consequence, hampered establishment can be considered a crucial factor for rarity of a species (e.g. Hitchmough, 2003; Hölzel and Otte, 2004; Overbeck et al., 2003). An example is the perennial Asteraceae *Arnica montana* L., one of the most endangered Central European grassland species. The species is handicapped by a low competitive ability (Pegtel, 1994) in combination with a high palatability for molluscs (Scheidel and Bruelheide, 1999, 2004a, b), which belong to the most important seedling consumers in grassland (e.g. Hulme, 1994, 1996). The palatability of *A. montana* might be the key factor for the species' montane distribution. In Central Europe, the harsh climatic conditions of montane sites can be assumed to restrict many unspecialized herbivores to lower elevations. In the lowlands, generalist invertebrates, particularly molluscs, may reach high densities due to their feeding on common food plants, and cause the local or regional extinction of highly palatable plant species if these are not able to tolerate the higher herbivory pressure in the lowlands (Bruelheide and Scheidel, 1999; Scheidel and Bruelheide, 2001; Scheidel et al., 2003). Thus, herbivory could be an alternative explanation to respiratory losses of storage carbohydrates, which are often considered the main cause for a lower elevational distribution boundary (Atkin et al., 1996; Bruelheide and Lieberum, 2001; Larigauderie and Körner, 1995; Scheidel and Bruelheide, 2004b; Stewart and Bannister, 1974).

To test the establishment success of *Arnica* seedlings under the artificially modified pressure of competition and herbivory, seeds were sown into vegetation-free gaps of two different sizes in a submontane meadow in the Harz Mountains, Central Germany, near to the species' regional lower altitudinal distribution limit. Removing the native vegetation may not only alter competitive conditions but also the seedlings' risk of mollusc herbivory (e.g. Davies et al., 2000; Hanley et al., 1996a; Overbeck et al., 2003). In addition, the herbivory pressure was reduced in half of the gaps by application of molluscicides. We hypothesized that the establishment rate assessed during the following two vegetation periods could be increased by creating larger gaps or protecting the gaps by molluscicides, and expected interaction effects of gap size and molluscicide application.

The effects of herbivory on *A. montana* were compared to a second montane Asteraceae species, *Centaurea pseudophrygia* C.A. Meyer. We hypothesized that *Centaurea* seedlings are better able to escape mollusc herbivory than the slow-growing *Arnica* seedlings due to the former's higher relative growth rate (Scheidel and Bruelheide, 2004a), which allows the *Centaurea* plants to compensate for the herbivory pressure. Another purpose of the experiments was to analyse the community response to mollusc exclusion, which revealed ambiguous results in the literature. We expected significant influences on the species number of seedlings emerging from the seedbank and differences in diversity. Therefore, in addition to investigating the seedling establishment of sown species, the general influence of herbivores on grassland species composition and diversity was tested by also recording all species emerging from the seedbank.

Materials and methods

The experiments were carried out in a submontane meadow located in a side valley of the Lutter River, 4 km north of Bad Lauterberg at the southern border of the Harz Mountains, Germany. The grassland of about 3 ha surrounded by beech and spruce forests is grazed by cattle once or twice per year in late summer. Experimental plots (10 m × 7.5 m for each of three *Arnica* experiments and 5 m × 7.5 m for the *Centaurea* experiment) were located on a northeast facing slope at about 400 m a.s.l., which is characterized by an inclination of 22–25° and a homogeneous vegetation. The vegetation was sampled on 15 July 1999 on a 2 m × 2 m square plot directly adjacent to the experimental plots (Table 1; nomenclature of vascular plant species after Ehrendorfer, 1973; mosses after Frahm and Frey, 1992). *A. montana* and *C. pseudophrygia* did not occur in this meadow. They were neither in its seedbank (categorized as transient by Kahmen and Poschlod, 1998; Thompson et al., 1997), nor were they expected to arrive naturally because of their low seed dispersal ability (Müller-Schneider, 1986, p. 204; Strykstra et al., 1998). The nearest small *Arnica* stand was located about 600 m away. The nearest *C. pseudophrygia* plants occurred at a distance of several kilometres. However, from the existing vegetation it can be assumed that the study site provides appropriate habitat conditions and regeneration niches comparable to montane meadows with existing populations of both species, without considering the herbivory pressure.

The first of three *Arnica* experiments started with gap creation and sowing in the spring, on 8 April 1999. To study seasonal effects on seedling establishment, a second experiment on a directly adjacent plot started in summer, on 12 August 1999. To assess interannual differences in seedling establishment, a third experiment was begun on a new plot next to the summer plot on 3 April of the following year.

Table 1. Vegetation relevé in a 2 m × 2 m plot adjacent to the sites of the *Arnica* and *Centaurea* sowing experiments in the Harz Mountains on 15 July 1999

Cover of herb layer	90%	Height of herb layer	40–80 cm
Cover of moss layer	20%	Cover of litter	40%
Open ground	1%		
Festuca rubra	20	*Ranunculus* sp.	2
Agrostis tenuis	10	*Potentilla erecta*	2
Anthoxanthum odoratum	4	*Anemone nemorosa*	2
Holcus lanatus	4	*Knautia arvensis*	2
Dactylis glomerata	4	*Leucanthemum vulgare*	2
Poa pratensis	1	*Rumex acetosa*	2
Meum athamanticum	30	*Cirsium palustre*	1
Hypericum maculatum	20	*Phyteuma nigrum*	1
Stellaria graminea	4	*Campanula rotundifolia*	1
Veronica chamaedrys	4	*Rhytidiadelphus* sp. M	20
Lotus uliginosus	4	*Plagiomnium affine* M	1
Galium harcynicum	2		

Note: Values are class means of a modified Londo (1975) scale. M = moss.

In each experiment, 48 circular gaps were created by cutting around the edge with a spade, removing the turf, breaking up the soil slightly and removing rhizomes and coarse roots. To assess the effects of gap size, the gaps had a diameter of 20 cm ('small') or 28 cm ('large'), similar to those of naturally occurring grassland gaps like mole hills, ant hills and larger down-slope cattle footsteps. Two small and two large randomly distributed gaps were combined for the molluscicide treatment into blocks. Within these sets the centres of the gaps were separated by 1 m; whereas a distance of 1.5 m separated them from the next gap of a neighbouring set. A total of 24 gaps (in six blocks with two small and two large gaps each) were chosen for mollusc exclusion. The remaining gaps served as unprotected controls. In each experiment, on 4–5 dates within about 10 weeks after sowing, 4 g of molluscicide pellets with a metaldehyde basis were spread in a perimeter band of 20 cm around each protected gap. To avoid possible direct molluscicide effects on the seedling germination or establishment (Gange et al., 1992; Hanley and Fenner, 1997), no pellets were spread into the gaps. Dead slugs were removed from the gaps. In this meadow, we found mainly slug individuals of *Arion subfuscus*, *A. rufus* and *Deroceras* sp. (mollusc nomenclature after Schaefer, 2002).

Arnica seeds for sowing had been collected in montane meadows in the Harz Mountains at about 600 m a.s.l., the year previous to the spring experiments, and some weeks previous to the summer experiment and had been stored dry and at room temperature. The smaller gaps were evenly sown with 25 *Arnica* seeds, the nearly two-fold larger gaps were sown with 50 *Arnica* seeds, thus attaining almost the same seed densities (796 or 812 seeds/m^2). After sowing, the soil was slightly pressed and watered to improve the soil contact of the seeds. Thereafter, the gaps were not watered to observe the seedling establishment under natural weather conditions.

Until the autumn of 2000, the plots were visited about every 10 days within the growing seasons to assess the number of *Arnica* plants in each gap. In addition, the numbers of monocotyledon and dicotyledon seedlings from the seedbank were counted until about 10 weeks after the beginning of the experiments. When most seedlings had developed at least one or two foliage leaves they were identified to a genus or species level wherever possible. To assess the α-diversity of the seedbank species the Shannon–Wiener index was calculated, including monocots and undetermined dicots as single taxa. Individuals that regenerated from root or rhizome fragments (mainly *Meum athamanticum*) or invaded from the surrounding vegetation (e.g. *Galium harcynicum*) were not recorded. During a period of about 10 weeks after gap creation, their above-ground parts were carefully removed and the surrounding vegetation that reached into the gaps was repeatedly cut.

The second study species, *C. pseudophrygia*, was sown in one spring experiment, starting on 10 April 2000. The seeds had been collected 1 year before in montane meadows at about 550 m a.s.l. in the Harz Mountains. The experimental plot of 5 m × 7.5 m was established directly adjacent to the plot of the *Arnica* experiment started in April 2000. In the *Centaurea* experiment, the effects of gap size were not tested. The 24 'large' gaps (diameter: 28 cm) were arranged in six sets of four gaps, with distances between the gaps as in the *Arnica* experiments. Twelve gaps were treated with molluscicides. In addition to the *Centaurea* seedlings, those of dicotyledons and monocotyledons from the seedbank were counted for about 10 weeks after beginning the experiment, at which time most dicots could be identified to a species level. The survival of the *Centaurea* plants was monitored until the end of the growing season.

Data analysis

Using the data of seedling densities without transformation, two-way analyses of variance were performed to determine the effects of mollusc exclosure and gap size on the seedling densities of *A. montana*, of monocotyledons and of dicotyledons on the date of the maximum *Arnica* seedling density, and on the seedling densities of *Arnica* at the end of the experiments in September 2000 for each of the three *Arnica* experiments. We employed a nested two-way ANOVA model testing the block effect (random) nested within molluscicide treatment and the effects of mollusc exclosure (fixed) and gap size (fixed). Combining all three *Arnica* experiments, a nested three-way ANOVA model was used to test the block effect (random) nested within molluscicide treatment and the interacting effects of mollusc exclosure, gap size and sowing time (all fixed) on the *Arnica* seedling density about 10 weeks after sowing. Additionally, a two-way ANOVA tested the effects of mollusc exclosure and plant species, comparing *Arnica* and *Centaurea* sown in the spring of 2000, on their seedling density on the date of maximum seedling density in June 2000, and again at the end of the experiments in September 2000. Effects of slug exclosure on the seedling density of *Centaurea* and the seedbank species in the *Centaurea* experiment were determined by one-way ANOVA. All calculations were performed with SAS (proc glm, SAS Institute, 2000).

Results

The first seedlings of *A. montana* emerged about 7 weeks after sowing on 8 April 1999, 2 weeks after sowing on 12 August 1999 and 5 weeks after sowing on 3 April 2000 (Fig. 1). The initial phases of the two spring experiments were characterized by very dry weather conditions. In spite of this, the germination of *C. pseudophrygia* started about 2 weeks after sowing on 10 April 2000 (Fig. 2).

In all three *Arnica* experiments, the establishment rate, calculated as percentage of sown seeds, reached its highest values in the small molluscicide-treated gaps (Fig. 1). In the first experiment, the maximum establishment rate in these gaps was found on 6 July at about 34% of sown seeds, corresponding to a density of 8.5 seedlings/gap or 270 seedlings/m^2, at 43% on 20 September after sowing in the summer of 1999, and at 30% on 16 June after sowing in the spring of 2000. Comparing the establishment rates of the three experiments on these dates, we found a highly significant effect of sowing time (Table 2). In the *Centaurea* experiment, the maximum establishment rate was 49% of sown seeds and encountered in the molluscicide-treated gaps on 16 June 2000 (Fig. 2). The seedling density of *Centaurea* was significantly higher than that of *Arnica* when only the large gaps in the parallel experiment in 2000 are considered (Table 3). This difference remained significant until the end of the study.

On the dates of maximum *Arnica* seedling densities, there was a considerable variability in establishment rates, particularly in the small molluscicide-treated gaps, between 4% and 84% of sown seeds after sowing in the spring of 1999, between 16% and 72% after sowing in the summer of 1999 and between 0% and 72% after sowing in the spring of 2000. In the *Centaurea* experiment, the establishment rate reached between 14% and 74% in the molluscicide-treated gaps.

Regarding the establishment rate on the date of its maximum, there was a highly significant difference in the seedling density between molluscicide-treated and unprotected gaps and also between small and large gaps, as well as a significant interaction in the *Arnica* experiment that started in the spring of 1999 (Table 4). In the *Arnica* experiments that started in the summer of 1999 and in the spring of 2000, a significant effect of mollusc exclosure, but no effect of gap size was found. A similar outcome was encountered in the *Centaurea* experiment where there was also a significant effect of slug exclosure. Seedling densities of *C. pseudophrygia* after 10 weeks were 272 and 401/m^2 in the control and molluscicide plots, respectively ($F = 6.12$, $p = 0.0215$).

Cattle grazing in July clearly reduced the numbers of the sown *Arnica* or *Centaurea* seedlings (Figs. 1 and 2). Slight increases later on were due to the regrowth of plants that were buried by cattle trampling for a time. In some cases, new seedlings germinated after cattle grazing. Subsequently, there were only minor changes until the end of the growing seasons. However, the survival data do not reflect the observation that most of the remaining *Arnica* plants suffered from severe herbivory damage to their leaves; whereas the survived *Centaurea* plants were less heavily damaged.

In the two experiments started in 1999, a clear over-winter decline was found for the *Arnica* seedling densities (Fig. 1). On the first sample date after winter, on 10 April 2000, *Arnica* plant densities were much lower than in November 1999. In the following weeks, further *Arnica* plants emerged from the litter layer. In addition, six seedlings germinated in some gaps that had been prepared in the summer of 1999. In May 2000, the mean *Arnica* survivorship rate of all treatment combinations in the first spring experiment reached 58% of the last sample in November 1999. The over-winter decline in the experiment that had started in the summer of 1999

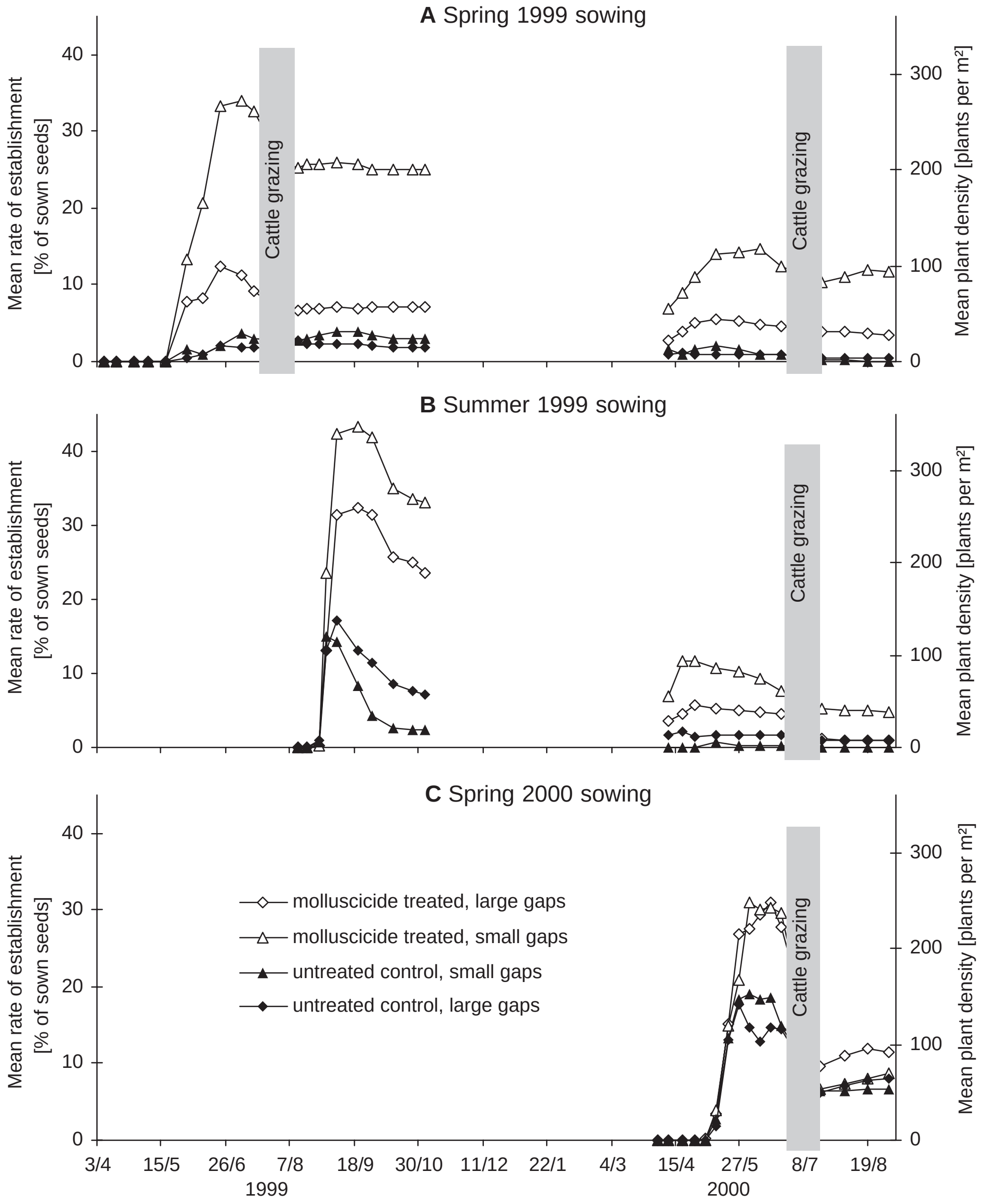

Fig. 1. Mean rate of establishment ($n = 12$) of *Arnica montana* seedlings as percentage of the number of sown seeds (left axis) and corresponding *Arnica* plant density (right axis) from the start of the experiments until 1 September 2000 in the large and small gaps, with and without mollusc exclusion. (A) Experiment started on 8 April 1999; (B) started on 12 August 1999; (C) started on 3 April 2000.

was even more dramatic. The observed herbivore damage to the new emerging shoots gave indications of mollusc herbivory as a major cause of this decline. The more vigorous plants of the first spring experiment were less vulnerable than the smaller ones sown in summer. However, these smaller plants were also observed to be more affected by frost heave.

In the experiment that had been started in the spring of 1999, the effect of slug exclosure on the *Arnica* seedling density remained significant until the end of the study (September 2000), although the molluscicide application was terminated in June 1999 (Table 4). In the experiments started on later dates, there remained no effect of initial mollusc exclosure by September 2000. Gap size had no effect on the *Arnica* density at the end of the study in any experiment.

In the two *Arnica* experiments started in 1999, both monocotyledons and dicotyledons originating from the seedbank reached the highest values in the small gaps with mollusc exclosure; whereas in the *Arnica* experiment started in the spring of 2000 they showed higher densities in the unprotected controls than in the

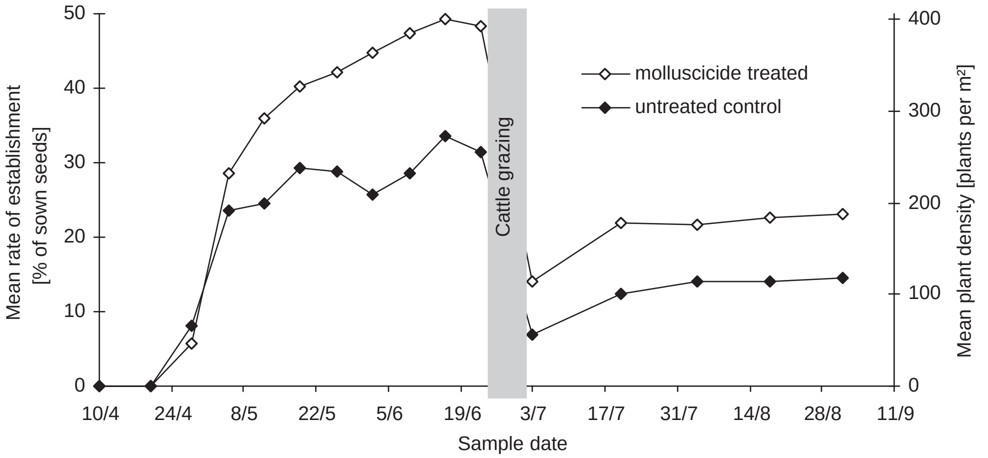

Fig. 2. Mean rate of establishment ($n = 12$) of *Centaurea pseudophrygia* seedlings as percentage of the number of sown seeds (left axis) and corresponding *Centaurea* plant density (right axis) from the beginning of the experiment on 10 April 2000 until 1 September 2000 in the gaps with and without mollusc exclusion.

Table 2. Results of three-way nested ANOVA on the effects of mollusc exclosure (*E*), gap size (*G*) and sowing time (*T*) on the seedling densities of *Arnica montana* on the dates of maximum *Arnica* seedling density

	d.f.	*SS*	*F*
E	1	962,036	27.99
Block (*E*)	10	343,732	2.32*
G	1	65,694	4.44*
T	2	263,644	8.90***
E × *G*	1	59,839	4.04*
E × *T*	2	65,642	2.22
G × *T*	2	52,655	1.78
E × *G* × *T*	2	70,466	2.38
Error	12	33,391	

*$P<0.05$; ***$P<0.001$.

Table 3. Results of two-way ANOVA on the effects of mollusc exclosure (*E*) and plant species (*S*; *Arnica montana* or *Centaurea pseudophrygia*) on the seedling densities of the sown species in the large gaps in the experiments started in the spring of 2000 on the date of maximum seedling density on 16 June 2000 and at the end of the experiments on 1 September 2000

	16 June 2000			1 September 2000		
	d.f.	*SS*	*F*	d.f.	*SS*	*F*
E	1	3136.3	13.70**	1	232.0	3.03
S	1	4181.3	18.27**	1	972.0	6.81*
E × *S*	1	1.3	0.01	1	75.0	0.53
Error	44	10,072.7		44	6278.0	

*$P<0.05$; **$P<0.01$.

protected gaps for both small and large gaps (Fig. 3). Whereas in the experiment started in the spring of 1999 mollusc exclosure had highly significant effects on the densities of both monocotyledons and dicotyledons; no significant exclosure effects were encountered in the experiments that started on later dates. The factor gap size was significant in all experiments for both monocotyledons and dicotyledons, with the exception of the *Arnica* experiment that started in the spring of 2000 where the monocotyledons did not show a significant gap-size effect (Table 4). Similarly, in the *Centaurea* experiment the densities of seedbank species showed no effects of molluscicide application. Mollusc exclosure and gap size had also no effect on the number of dicot taxa (6.5/gap, on average, including the undetermined species as one taxon, in the first *Arnica* experiment; 4.8/gap in the second; and 6.8/gap in the third experiment) or on α-diversity (mean Shannon–Wiener index of 1.51 in the first experiment; 1.01 in the second; and 1.56 in the third experiment). In the *Centaurea* experiment, the number of dicot taxa per gap (8.0, on average), and the Shannon–Wiener index (1.68, on average) showed also no significant treatment effects.

Hypericum maculatum was the most frequently established dicot species in the two experiments started in 1999 (Fig. 4). In the experiments with *Arnica* or *Centaurea* started in 2000, the most frequently observed dicot species was *Potentilla erecta*. However, there was no single species for which significant differences between treatments were detected.

Discussion

This study is yet another confirmation of the immense impact that mollusc herbivory may exert on plant species establishment in grasslands, mainly by consuming seedlings. Because seedlings can be severely damaged

Table 4. Results of two-way nested ANOVA on the effects of mollusc exclosure (E) and gap size (G) on the seedling densities of *Arnica montana* and monocots and dicots from the seedbank on the date of maximum *Arnica* seedling density in each experiment, and on the seedling densities of *Arnica* at the end of the experiment on 1 September 2000

	d.f.	Sample date at maximum *Arnica* seedling density, 6–12 weeks after sowing						1 September 2000	
		Arnica montana		Monocotyledons		Dicotyledons		*Arnica montana*	
		SS	*F*	*SS*	*F*	*SS*	*F*	*SS*	*F*
Spring experiment 1999, started on 8 April 1999									
E	1	304,360	10.67**	1,533,847	31.70***	1,727,245	15.97**	40,260	6.56*
Block (E)	10	285,375	3.68**	483,850	0.36	1,081,761	1.90	61,374	1.34
G	1	111,538	14.40***	1,935,688	14.52***	2,558,210	44.97***	11,425	2.49
$E \times G$	1	80,922	10.44**	556,869	4.18*	134,875	2.37	14,629	3.19
Error	34	263,434		4,531,946		1,934,266		155,754	
Summer experiment 1999, started on 12 August 1999									
E	1	586,979	15.68**	81,673	0.39	25,977	0.15	4444	1.87
Block (E)	10	362,850	2.76*	2,107,642	1.11	171,063	4.22***	23,711	1.06
G	1	5555	0.42	1,681,382	8.89**	390,034	9.62**	1485	0.66
$E \times G$	1	44,306	3.37	1421	0.01	15,294	0.38	3840	1.72
Error	34	12,476		6,427,930		1,378,420		76,079	
Spring experiment 2000, started on 3 April 2000									
E	1	154,339	5.93*	214,274	1.49	282,924	3.15	5896	0.80
Block (E)	10	260,095	1.66	1,442,221	2.89*	899,135	1.19	73,288	1.14
G	1	12,556	1.66	166,714	3.34	1,638,145	21.61***	3959	0.62
$E \times G$	1	5078	0.32	13,451	0.27	30,839	0.41	469	0.07
Error	34	531,616		1,697,369		2,577,170		6423	

*$P<0.05$; **$P<0.01$; ***$P<0.001$.

by comparatively few bites, they are the stage of the plant's life cycle most vulnerable to herbivory (Crawley, 1987; Harper, 1977; Fenner, 1987). The risk of being consumed is, however, not identical for seedlings of different plant species. Observational and experimental work has shown that slugs and snails attack in a species-specific manner and with varying consequences (e.g. Dirzo and Harper, 1980; Hulme, 1994, 1996). As shown by Fenner et al. (1999) and Wardle et al. (1998), discrimination among seedlings of different species is only weakly or not at all correlated with the species-specific palatability found in adult material. Although leaf discs of *H. maculatum* were found to be relatively unpalatable for slugs (Scheidel and Bruelheide, 1999), this species tended to profit from mollusc exclosure in our experiments. *Hypericum* may suffer from herbivory because its very small seedlings could be easily consumed. Similarly, mature leaves of grasses are generally considered to be avoided by molluscs (e.g. Briner and Frank, 1998; Hanley et al., 1996b). In contrast, our first experiment showed a significantly positive effect of mollusc exclosure on the seedling establishment of monocotyledons.

The time a seedling needs to leaves this most vulnerable stage to reach the stage of herbivory risk associated with mature plants depends on the plant species and the herbivores involved. Several authors found a decreasing mortality after mollusc herbivory with increasing plant age. In a transplantation experiment with 3-week-old seedlings of three grassland species (Reader, 1992), the majority of death events, mainly due to herbivory, occurred within 2–4 weeks after transplantation, depending on the plant species. Seedling age, differing only by 14 days in the experiments by Hanley et al. (1995b), had a marked effect on the likelihood of attack and mortality in three grassland species. Scheidel and Bruelheide (2004a) found a decreasing mortality rate of *C. pseudophrygia* seedlings with increasing age (1–5 weeks) on the date of transplantation into a slug-rich meadow. In laboratory and field experiments by Fritz et al. (2001), mollusc damage to willow seedlings decreased drastically from week 7 to week 9.

Besides the fact that from older, larger plants more tissue has to be consumed to kill them, there are many plant traits changing with age that may influence palatability (e.g. Fritz et al., 2001; Linhart and Thompson, 1995). Both mechanisms to reduce the damaging effects of herbivory, growth and chemical defence, were suggested as alternative strategies in the struggle against herbivores (Herms and Mattson, 1992). Wardle et al. (1998) found that species that invest more

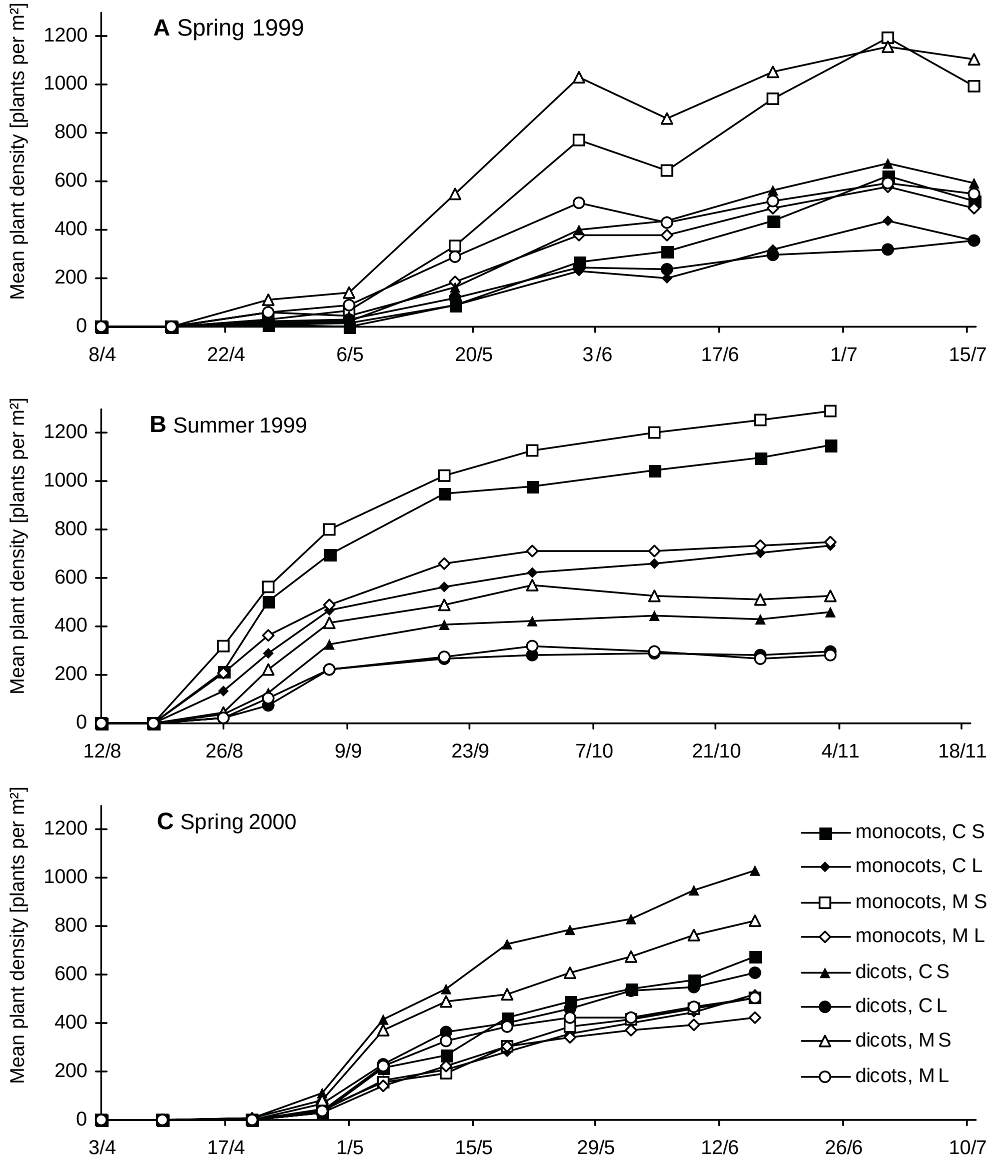

Fig. 3. Mean seedling density ($n = 12$) of monocots and dicots from the seedbank for a period of about 10 weeks after the beginning of the experiments in the large (L) and small (S) gaps, with mollusc exclusion (M) and without (control, C). (A) Experiment started on 8 April 1999; (B) started on 12 August 1999; (C) started on 3 April 2000.

in above-ground biomass were more palatable to slugs. In this respect, *A. montana* is doubly handicapped by a high palatability (Scheidel and Bruelheide, 1999) and a slow growth rate, resulting in a long lasting, highly sensitive seedling stage of several weeks. Subsequently, however, there were only slight reductions in the *Arnica* seedling density during the vegetation period, except of the effects of cattle trampling. Therefore, the significant molluscicide effect 3 months after sowing remained visible without further molluscicide application until the end of the experiment after 17 months. However, to fully demonstrate that factors exerting their effects on later life stages are less important than seedling herbivory the plants should ideally be monitored for herbivore damage until the reproductive stage, which needs at least three vegetation periods in *Arnica*.

This would have been also desirable for the seedbank species which could, however, be observed for only about 10 weeks, because after this period it became impossible to distinguish between seedlings and vegetative ramets in some species. Another difficulty for assessing the treatment effects on the seedbank species is their presumably high variability in seed number among the gaps as this factor had not been controlled in our experiment. This results in a lower probability to find significant herbivory effects compared to the sown species, although the seedling densities of the most frequently occurring seedbank species were in the same

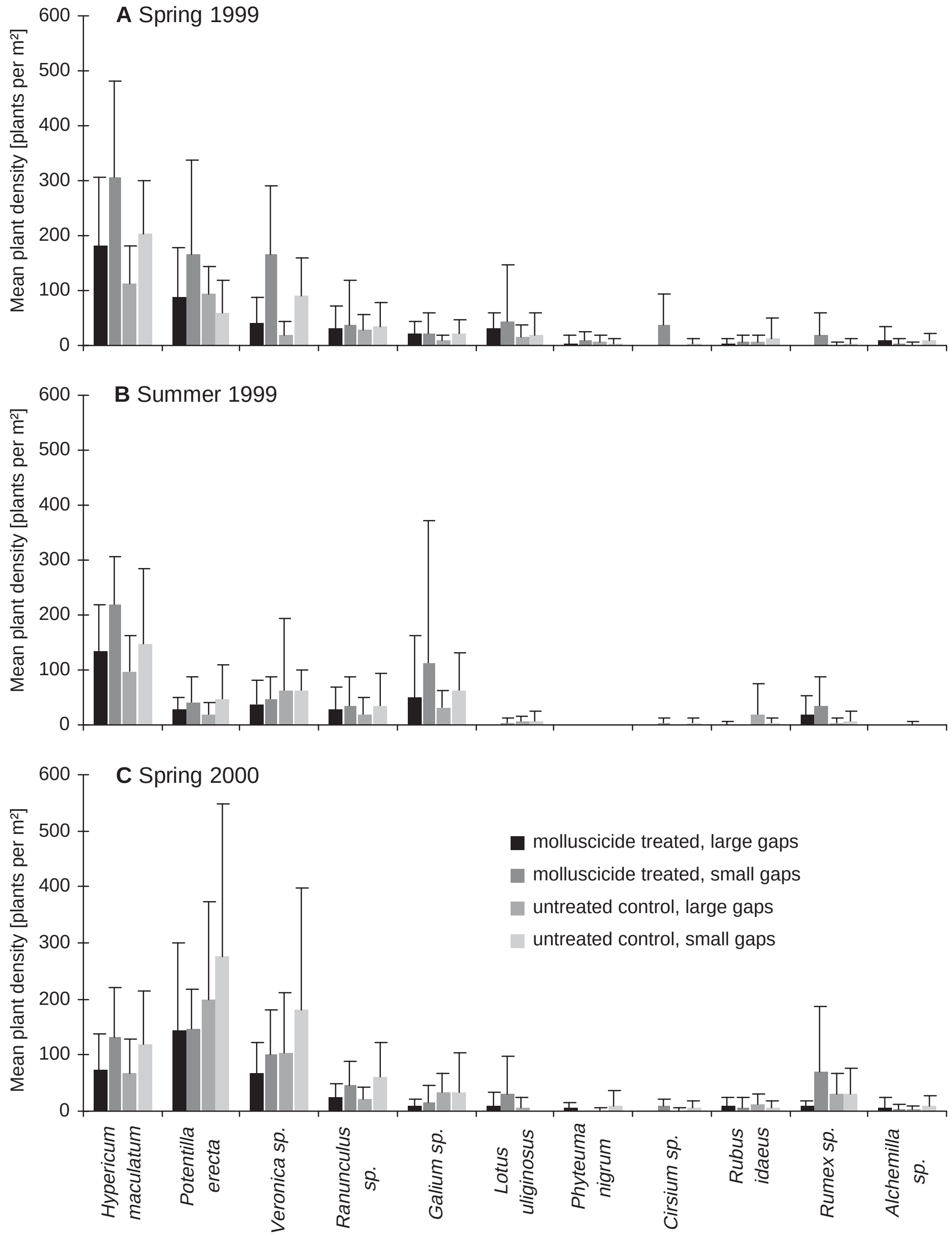

Fig. 4. Mean seedling density ($n = 12$) of the most frequently established dicot species from the seedbank about 10 weeks after the beginning of the experiments in the large and small gaps, with and without mollusc exclusion. (A) Experiment started on 8 April 1999; (B) started on 12 August 1999; (C) started on 3 April 2000. Error bars indicate standard deviation.

order of magnitude as those of the sown species. Consequently, molluscicide effects on single species of local origin or at the community level could not be detected in this short period. All this explains some of the discrepancy to other studies. For example, Wilby and Brown (2001) found a lower species richness after about 2 years of mollusc exclusion from abandoned grassland plots.

A further reason for the failure of herbivory effects on the seedbank species may be that the local species pool has been selected over long periods with respect to herbivory resistance. Small-scale dynamics are tightly linked to the species pool (e.g. Pärtel et al., 1996; Zobel, 1997; Zobel et al., 2000). Therefore, we cannot expect to find any sensitive species when excluding molluscs over some weeks or even years. Clear effects on the community level can therefore be found only with experimentally modified species pools, as, e.g. in the case of our sown species or in microcosm experiments (e.g. Diaz et al., 1998; Fraser and Grime, 1999). For a more conclusive comparison, other species than the target species have additionally to be sown into gaps. With respect to the lower altitudinal range limit of montane plant species, it would be very interesting to compare montane species with the close relatives of different range types, as it was done by Scheidel and Bruelheide (2001), who quantified the herbivore damage to 1-year-old transplanted individuals of three species pairs. Similarly, Scheidel and Bruelheide (2004a) assessed the damage to different-aged seedlings of the montane *C. pseudophrygia* and the common lowland relative *C. jacea*. However, the latter experiment revealed no clear differences in herbivory damage between the two species that would explain their contrasting distribution ranges. Thus, herbivory is probably not an all-purpose explanation for explaining lower elevational range boundaries.

The other manipulated factor in our experiment, gap size, may influence the seedling establishment through direct effects of different microclimates on germination (e.g. Watt, 1987), and through indirect effects on herbivore activity. In larger gaps, a smaller proportion of the area is shaded by the surrounding vegetation, and therefore more light, but a lower air and soil humidity, can be expected. Therefore, in our experiments, the smaller gaps possibly offered a more appropriate microclimate for germination during the dry periods immediately after gap creation. In most cases, more seedlings per unit area became established in the smaller gaps. Although the establishment of the sown *Arnica* was influenced by gap size only in the first spring experiment, monocotyledons were affected by gap size after sowing in August as well, and dicots were affected in all three experiments, with a significant interaction between gap size and slug exclosure in the first spring experiment for *Arnica* and monocots. This could be an indication of increasing slug predation with increasing gap diameter. An explanation might be a greater attraction of large gaps with larger amounts of palatable seedlings to slugs (Davies et al., 2000). The effect of increasing losses of seedlings to slug herbivory in the largest gaps was also described by Hitchmough (2003) for establishment experiments with the montane grassland species *Trollius europaeus*. Hanley et al. (1996a) found a significant effect of gap size on overall densities of seedlings sown into gaps with a diameter of 15 or 30 cm and species-specific differences. However, the authors found no interaction between effects of gap size and molluscicide application. In this study, gap-size effects were obviously caused by external factors, such as microclimate. Hanley et al. (1996b) found an interaction between mollusc exclusion and gap size for only one or two species germinating from the seedbank in a spring and autumn experiment. The seedling density was greater in the smaller (15 cm diameter) than in the larger (30 cm) gaps. In the long run, however, stronger competition with the invading surrounding vegetation might reduce the seedling establishment to a greater extent in smaller gaps.

The sequence of *Arnica* sowing experiments started in the spring and late summer of 1999 and in the spring of the following year revealed no clear differences in the long-term establishment success between years or seasons, although a significant time effect was found in the comparison of establishment rates 10 weeks after sowing. In contrast, Hanley et al. (1995a, 1996a, b) and Hulme (1994) found clear seasonal effects on the seedling survivorship of grassland species because mollusc populations were greater in autumn than in spring. Therefore, Hulme (1994) discussed the predominant germination of grassland species in the spring as an escape mechanism to avoid herbivory. However, in Central Europe, seasonal differences in the mollusc activity found in one or a few study years may actually be weather-dependent and non-periodical, resulting in a pronounced interannual variability (e.g. Nystrand and Granstrom, 2000; South, 1989; Voss et al., 1998). Therefore, it cannot be ruled out that the low unprotected establishment of *Arnica* found in all three experiments may be followed by exceptional years with low mollusc activity, resulting in a higher establishment success. In general, it is still an open question how frequent the successful regeneration by seedlings must be to sustain viable populations of long-lived perennials with slow clonal growth, a category to which belong *A. montana* and *C. pseudophrygia* according to the database of Klimeš et al. (1997). Rare stochastic events of herbivore-free regeneration niches may be sufficient to sustain these populations in the long run.

For the years and seasons under investigation, seedling establishment of *A. montana* at the experimental site, a meadow at the lower altitudinal range limit of the species, can be considered impossible because of high mollusc herbivory. *C. pseudophrygia* was able to cope with slug herbivory to a slightly higher degree; after one growing season the surviving young plants were much larger than the *Arnica* plants of the same age, and could therefore be expected to tolerate a larger amount of herbivore consumption in the future.

For this experiment, we have no comparison to experimental plots at higher or lower altitudes yet. At the elevation of the study site, the herbivory damage to adult *Arnica* plants found in a native population at a distance of some hundred metres is low. Transplanted 1-year-old *Arnica* individuals suffered a leaf area loss of less than 10% (Bruelheide and Scheidel, 1999). This is in the range of damage reported from many plant species and communities (Crawley, 1989; Pimentel, 1988; Sand-Jensen et al., 1994). In contrast, *Arnica* seedlings transplanted into a lowland meadow were completely consumed by molluscs within a few days (Scheidel and Bruelheide, 2004a). From the experiments with 1-year-old *Arnica* individuals (Bruelheide and Scheidel, 1999) and further Asteraceae species (Scheidel and Bruelheide, 2001) we predict a generally decreasing herbivore pressure with increasing altitude also for seedlings. Along the altitudinal gradient from montane sites to the lower range limit, seedling establishment should become impossible even at sites where adult plants could potentially persist.

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References

Atkin, O.K., Botman, B., Lambers, H., 1996. The causes in inherently slow growth in alpine plants: an analysis based on the underlying carbon economies of alpine and lowland *Poa* species. Funct. Ecol. 10, 698–707.

Briner, T., Frank, T., 1998. The palatability of 78 wildflower strip plants to the slug *Arion lusitanicu*s. Ann. Appl. Biol. 133, 123–133.

Bruelheide, H., Lieberum, K., 2001. Experimental tests for determining the causes of the altitudinal distribution of *Meum athamanticum* Jacq. in the Harz Mountains. Flora 196, 227–241.

Bruelheide, H., Scheidel, U., 1999. Slug herbivory as a limiting factor for the geographical range of *Arnica montana*. J. Ecol. 87, 839–848.

Crawley, M.J., 1987. Herbivores and plant population dynamics. In: Davy, A.J., Hutchings, M.J., Watkinson, A.R. (Eds.), Plant Population Ecology. Symp. Br. Ecol. Soc. 28, 367–392. Blackwell, Oxford.

Crawley, M.J., 1989. Insect herbivores and plant population dynamics. Ann. Rev. Entomol. 34, 531–564.

Davies, A., Dunnett, N.P., Kendle, A.D., 2000. The importance of transplant size and gap width in the botanical enrichment of species-poor grasslands in Britain. Rest. Ecol. 7, 271–280.

Diaz, S., Fraser, L.H., Grime, J.P., Falczuk, V., 1998. The impact of elevated CO_2 on plant-herbivore interactions: experimental evidence of moderating effects at the community level. Oecologia 117, 177–186.

Dirzo, R., Harper, J.L., 1980. Experimental studies on slug-plant interactions II. The effect of grazing by slugs on high density monocultures of *Capsella bursa-pastoris* and *Poa annua*. J. Ecol. 68, 999–1011.

Ehrendorfer, F., 1973. Liste der Gefäßpflanzen Mitteleuropas, second ed. Fischer, Stuttgart.

Fenner, M., 1987. Seedlings. New Phytol. 106 (Suppl.), 35–47.

Fenner, M., Hanley, M.E., Lawrence, R., 1999. Comparison of seedling and adult palatability in annual and perennial plants. Funct. Ecol. 13, 546–551.

Frahm, J.-P., Frey, W., 1992. Moosflora, third ed. Ulmer, Stuttgart.

Fraser, L.H., Grime, J.P., 1999. Interacting effects of herbivory and fertility on a synthesized plant community. J. Ecol. 87, 514–525.

Fritz, R.S., Hochwender, C.G., Lewkiewicz, D.A., Bothwell, S., Orians, C.M., 2001. Seedling herbivory by slugs in a willow hybrid system: developmental changes in damage, chemical defense, and plant performance. Oecologia 129, 87–97.

Gange, A.C., Brown, V.K., Farmer, L.M., 1992. Effects of pesticides on the germination of weed seeds: implications for manipulative experiments. J. Appl. Ecol. 29, 303–310.

Hanley, M.E., Fenner, M., 1997. Effects of molluscicides on seedlings of four grassland plant species. J. Appl. Ecol. 34, 1479–1483.

Hanley, M.E., Fenner, M., Edwards, P.J., 1995a. An experimental field study of the effects of mollusc grazing on seedling recruitment and survival in grassland. J. Ecol. 83, 621–627.

Hanley, M.E., Fenner, M., Edwards, P.J., 1995b. The effect of seedling age on the likelihood of herbivory by the slug *Deroceras reticulatum*. Funct. Ecol. 9, 754–759.

Hanley, M.E., Fenner, M., Edwards, P.J., 1996a. Mollusc grazing and seedling survivorship of four common grassland plant species: the role of gap size, species and season. Acta Oecol. 17, 331–341.

Hanley, M.E., Fenner, M., Edwards, P.J., 1996b. The effect of mollusc grazing on seedling recruitment in artificially created grassland gaps. Oecologia 106, 240–246.

Harper, J.L., 1977. Population Biology of Plants, first ed. Academic Press, London.

Herms, D.A., Mattson, W.J., 1992. The dilemma of plants: to grow or to defend. Q. Rev. Biol. 67, 283–335.

Hitchmough, J.D., 2003. Effects of sward height, gap size, and slug grazing on emergence and establishment of *Trollius europaeus* (globeflower). Rest. Ecol. 11, 20–28.

Hölzel, N., Otte, A., 2004. Ecological significance of seed germination characteristics in flood-meadow species. Flora 199, 12–24.

Hulme, P.E., 1994. Seedling herbivory in grassland: relative impact of vertebrate and invertebrate herbivores. J. Ecol. 82, 873–880.

Hulme, P.E., 1996. Herbivores and the performance of grassland plants: a comparison of arthropod, mollusc and rodent herbivory. J. Ecol. 84, 43–51.

Kahmen, S., Poschlod, P., 1998. Untersuchungen zu Schutzmöglichkeiten von Arnika (*Arnica montana* L.) durch Pflegemaßnahmen. Jahrb. Natursch. Hessen 3, 225–232.

Klimeš, L., Klimešová, J., Hendriks, R., van Groenendael, J., 1997. Clonal plant architectures: a comparative analysis of form and function. In: de Kroon, H., van Groenendael, J. (Eds.), The Ecology and Evolution of Clonal Plants. Backhuys Publishers, Leiden, pp. 1–29.

Larigauderie, A., Körner, C., 1995. Acclimation of leaf dark respiration to temperature in alpine and lowland plant species. Ann. Bot. 76, 245–252.

Linhart, Y.B., Thompson, J.D., 1995. Terpene-based selective herbivory by *Helix aspersa* (Mollusca) on *Thymus vulgaris* (Labiatae). Oecologia 102, 126–132.

Londo, G., 1975. Dezimalskala für die vegetationskundliche Aufnahme von Dauerquadraten. In: Schmidt, W. (Ed.), Sukzessionsforschung. Ber. Int. Symp. Int. Vereinig. Vegetationsk. Rinteln 1973, 613–617.

Müller-Schneider, P., 1986. Verbreitungsbiologie der Blütenpflanzen Graubündens. Veröff. Geobot. Inst. ETH Zürich 85, 1–263.

Nystrand, O., Granstrom, A., 2000. Predation on *Pinus sylvestris* seeds and juvenile seedlings in Swedish boreal forest in relation to stand disturbance by logging. J. Appl. Ecol. 37, 449–463.

Overbeck, G., Kiehl, K., Abs, C., 2003. Seedling recruitment of *Succisella inflexa* in fen meadows: importance of seed and microsite availability. J. Veg. Sci. 6, 97–104.

Pärtel, M., Zobel, M., Zobel, K., Van der Maarel, E., 1996. The species pool and its relation to species richness – evidence from Estonian plant communities. Oikos 75, 111–117.

Pegtel, D.M., 1994. Habitat characteristics and the effect of various nutrient solutions on growth and mineral nutrition of *Arnica montana* L. grown on natural soil. Vegetatio 114, 109–121.

Pimentel, D., 1988. Herbivore population feeding pressure on plant hosts: feedback evolution and host conservation. Oikos 53, 289–302.

Reader, R.J., 1992. Herbivory, competition, plant mortality and reproduction on a topographic gradient in an abandoned pasture. Oikos 65, 414–418.

Sand-Jensen, K., Jacobsen, D., Duarte, C.M., 1994. Herbivory and resulting plant damage. Oikos 69, 545–549.

SAS Institute, 2000. SAS Procedures Guide, version 8. SAS Institute, Cary, NC.

Schaefer, M., 2002. Brohmer, Fauna von Deutschland, 21th ed. Quelle & Meyer, Heidelberg-Wiesbaden.

Scheidel, U., Bruelheide, H., 1999. Selective slug grazing on montane meadow plants. J. Ecol. 87, 828–838.

Scheidel, U., Bruelheide, H., 2001. Altitudinal differences in herbivory on montane Compositae species. Oecologia 129, 75–86.

Scheidel, U., Bruelheide, H., 2004a. Age-specific and season-specific mollusc damage to seedlings of grassland Asteraceae. J. Torr. Bot. Soc. 131, 140–149.

Scheidel, U., Bruelheide, H., 2004b. The impact of altitude and simulated herbivory on the growth and carbohydrate storage of *Petasites albus*. Plant Biol. 6, 740–745.

Scheidel, U., Röhl, S., Bruelheide, H., 2003. Altitudinal gradients of generalist and specialist herbivory on three montane Asteraceae. Acta Oecol. 24, 275–283.

South, A., 1989. The effect of weather and other factors on numbers of slugs on permanent pasture. In: Henderson, I.F. (Ed.), Slugs and Snails in World Agriculture. Br. Crop Prot. Council Monogr. 41, 355–360. Thornton Heath.

Stewart, W.S., Bannister, P., 1974. Dark respiration rates in *Vaccinium* ssp. in relation to altitude. Flora 163, 415–421.

Strykstra, R.J., Pegtel, D.M., Bergsma, A., 1998. Dispersal distance and achene quality of the rare anemochorous species *Arnica montana* L.: implications for conservation. Acta Bot. Neerl. 47, 45–56.

Thompson, K., Bakker, J.P., Bekker, R.M., 1997. Soil Seed Banks of North West Europe: Methodology, Density and Longevity. University Press, Cambridge.

Voss, M.C., Hoppe, H.-H., Ulber, B., 1998. Estimation of slug activity and slug abundance. Z. Pflanzenkr. Pflanzenschutz 105, 314–321.

Wardle, D.A., Barker, G.M., Bonner, K.I., Nicholson, K.S., 1998. Can comparative approaches based on plant ecophysiological traits predict the nature of biotic interactions and individual plant species effects in ecosystems? J. Ecol. 86, 405–420.

Watt, T.A., 1987. Establishment of *Senecio jacobaea* L. from seed in grassland and in boxed swards. Weed Res. 27, 259–266.

Wilby, A., Brown, V.K., 2001. Herbivory, litter and soil disturbance as determinants of vegetation dynamics during early old-field succession under set-aside. Oecologia 127, 259–265.

Zobel, M., 1997. The relative role of species pools in determining plant species richness – an alternative explanation for species coexistence? Trends Ecol. Evol. 12, 266–269.

Zobel, M., Otsus, M., Liira, J., Moora, M., Möls, T., 2000. Is small-scale species richness limited by seed availability or microsite availability? Ecology 81, 3274–3282.