

The importance of nitrogen and carbohydrate storage for plant growth of the alpine herb *Veratrum album*

David Kleijn^{1,2}, Urs A. Treier¹ and Heinz Müller-Schärer¹

¹Department of Biology, Unit of Ecology & Evolution, University of Fribourg, Chemin du Musée 10, CH–1700 Fribourg, Switzerland; ²Present address: Nature Conservation and Plant Ecology Group, Wageningen University, Bornsesteeg 69, NL–6708 PD, Wageningen, the Netherlands

Summary

- We examined whether nitrogen (N) and carbohydrates reserves allow *Veratrum album*, an alpine forb, to start spring growth earlier than the neighbouring vegetation and to survive unpredictable disturbances resulting in loss of above-ground biomass.
- Seasonal dynamics of plant reserves, soil N availability and vegetation growth were monitored. *Veratrum album* shoots were experimentally removed when carbohydrate reserves were at a seasonal minimum and the subsequent changes in biomass and reserves were compared with those in control plants.
- Reserves did not give *V. album* a competitive advantage in spring; however, they did function as a buffer against the impact of calamities. Shoot removal resulted in significantly lower root dry weight, higher N concentration in rhizome and roots and lower starch concentrations in rhizome and roots but no plant mortality was observed.
- *Veratrum album* used stored N reserves to supplement N uptake and establish high leaf N concentrations, which facilitated a rapid refilling of depleted carbohydrate reserves. The primary function of N reserves appears to be to allow *V. album* to complete the growing cycle in as short a period as possible, thus minimizing exposure to above-ground risks.

Key words: carbohydrates, competition, defoliation, Liliaceae, nitrogen (N), risk aversion, reserves, storage.

Introduction

Many perennial plants store large quantities of nitrogen (N) and carbohydrates rather than use them directly for growth. These reserves may serve two important functions. First, stored resources may give plants a competitive advantage, for example, if it allows them to start spring-growth before their neighbours do (Heilmeyer *et al.*, 1986). Second, resources may be stored to bridge temporal gaps that exist between resource availability and resource demand (Bloom *et al.*, 1985; Chapin *et al.*, 1990). A distinction can be made between predictable temporal gaps such as seasonal periods of cold or drought (Boyce & Volenec, 1992; Wyka, 1999) and unpredictable gaps caused by calamities such as herbivory, shading or frost (van der Heyden & Stock, 1996; Kobe, 1997).

These potential functions of stored reserves may be particularly relevant to alpine plant species. Alpine habitats are characterized by long, cold winters, short growing seasons and the irregular occurrence of calamities destroying part or all above-ground biomass (e.g. late spring frost). Reserves might contribute significantly to a plant's fitness under the harsh growing conditions at high altitude (Bloom *et al.*, 1985; Körner, 1999). However, a comparison of species growing exclusively at low-altitude habitats with similar species growing exclusively at high-altitude habitats showed no difference in the quantity of below-ground storage organs (Körner & Renhardt, 1987). This suggests that the success of perennial herbaceous plants at high elevations does not necessarily depend on large below-ground stored resources.

The present study examines the significance of stored reserves for the vegetative success of *Veratrum album* (Liliaceae), a species

that invests heavily in reserves. Individual *V. album* plants flower infrequently and vegetative plants provide a relatively simple study system with respect to N and nonstructural carbohydrates storage and allocation through the season. Plants have a single rhizome and usually produce a single preformed shoot per year. Roots are thick, conspicuous and can therefore easily be distinguished from roots of other species. These traits facilitate an accurate determination of the seasonal dynamics of reserves and interpretation of the results. In central Europe *V. album* grows above 800 m elevation in grasslands and open forests. In these habitats, *V. album* is one of a group of tall, unpalatable forbs that have large below-ground storage organs (e.g. *Cirsium* spp., *Gentiana lutea*, *Rumex alpinus* and *Senecio alpinus*). Its rhizome serves as a storage organ and contains high concentrations of carbohydrates and N (Schaffner *et al.*, 1995).

Both above-mentioned functions of reserves may be highly relevant to *V. album*. First, the provision of an early season competitive advantage is important as *V. album* seems to be a poor competitor for nutrients. *V. album* has a coarse root system without ramifications and with no or limited root hairs. In general, specific root length and branching intensity are considered to be positively related to the efficiency of exploration and exploitation of mobile soil resources (Fitter, 1987). Associations with mycorrhizal fungi generally enhance the capability to capture nutrients of plants with coarse root systems but it is unknown whether *V. album* grows in association with such fungi. *Veratrum album* grows in habitats that are characterized by permanent snow cover during winter and a brief but predictable peak in N availability right after snow melt (Jaeger & Monson, 1992; Lipson *et al.*, 1999). Stored resources may allow *V. album* to start growth earlier than competing neighbouring plants, thus providing the species with a well-timed competitive advantage when nutrient availability is high.

Second, reserves as an insurance against unpredictable calamities may be important as, similar to many other Liliaceae species, *V. album* does not compensate for any loss of above-ground plant parts within a growing season. A damaged individual needs to rely on its reserves to survive until the next growing season. Reserves may thus be more important in Liliaceae species than in other species as insurance against calamities such as late frost, trampling, landslides or mowing. Carbohydrate reserves need to be sufficiently large to sustain maintenance respiration for the better part of a year.

We examined temporal variations in reserves in shoots, rhizomes and roots for one complete growth cycle and related this to temporal variations in N availability in the soil and growth of competing vegetation. In this way, we aimed to determine whether stored reserves allow *V. album* to start spring growth earlier than the neighbouring vegetation. Furthermore, we experimentally removed *V. album* shoots when reserves in rhizomes and roots were at a minimum to study the effects of calamities on plant growth and reserve formation in the remainder of the growing season. Thus, we evaluated

short-term consequences of calamities on plant growth and survival.

Materials and Methods

Species and field site

Veratrum album L. (Liliaceae) plants typically consist of a vertical rhizome supporting several bands of roots and bearing a single 20–60 cm tall vegetative shoot. Clonal reproduction may occur after flowering as the apical meristem dies and is replaced by two or three lateral buds. Initially, this results in plants with branched rhizomes and up to three shoots, but shoots become eventually separated as the connecting rhizome decays with time (Kleijn & Steinger, 2002). Plants must have a minimum size to be able to flower, but even large plants flower only every 4–8 yr. *Veratrum album* contains high concentrations of alkaloids, rendering them unpalatable to most herbivores. For more details regarding the life-history and ecology of the species see Schaffner *et al.* (2001).

We selected a population of *V. album* near Sangernboden in the Swiss Pre-Alps (46°40'4", 7°21'32", 1370 m a.s.l.) on a north-north-west facing slope. *Festuca rubra*, *Agrostis capillaris*, *Polygonum bistorta* and *V. album* dominated the vegetation. Soil type was a dystric cambisol and the field was used for summer grazing of cattle.

Studying temporal variations in reserves

In September 1999 we selected *V. album* plants by placing marker stakes near the shoot base. At this time shoots had senesced completely but were still easily recognizable. We selected single shoots that stood well apart from other shoots to ensure we included only plants with one shoot (size standardization) and to avoid using plants whose root system was entangled with that of conspecifics. The stakes allowed us to relocate the plants when shoots had decayed completely or when the study site was covered by snow.

The entire experiment was fenced during the growing season to exclude cattle. To compensate for environmental variation within the study site, we established four replicated blocks each approx. 10 × 20 m. Within each block, we randomly assigned 10 sampling dates to the marked individuals, with each sampling date being replicated twice. The sampling schedule was basically designed to cover an entire year (September 1999–July 2000), however, the sampling frequency was intensified in early spring, around snowmelt, as in this period the most significant quantitative changes in stored reserves are expected to occur.

Studying the effects of calamities on reserves

We applied two treatments, 'shoot removal' and 'vegetation removal'. The shoot removal treatment consisted of removing

the entire *V. album* shoot at ground level, thus simulating a worst case calamity. The vegetation removal treatment consisted of cutting all vegetation at ground level within a 25-cm radius of the *V. album* shoot base repeatedly throughout the growing season but leaving the *V. album* shoot intact. This treatment simulates livestock grazing, as in pastures *V. album* is not grazed while the competing vegetation is. The plants that were sampled to study temporal variations in reserves served as control plants; both *V. album* shoots and surrounding vegetation were left intact. A comparison of these controls with the vegetation removal treatment may indicate whether grazing releases the unpalatable *V. album* from above-ground competition by the neighbouring vegetation.

The shoot removal treatment was applied on 23 May 2000. At this time all *V. album* shoots had emerged and were beginning to unfold their leaves (phenological stage of the vegetation; *Soldanella alpina* and *Primula elatior* had just finished flowering and *Ranunculus aconitifolius* was in full bloom). Some time after shoot emergence, when growth is most rapid, geophytes generally experience a seasonal low in below-ground reserves (Chapin *et al.*, 1990; Schaffner *et al.*, 1995; Lipson *et al.*, 1996) and defoliation will then have the most pronounced impact. The vegetation removal treatment was first applied on 23 May 2000 and was repeated until final harvest at intervals of approx. 3 wk. The effects of the treatments were measured after 2 wk and 8 wk by sampling two plants of each treatment in each block. The study design was therefore a randomized complete block design with two sampling-date-treatment combinations nested in each block.

Veratrum album sampling and analysis

At each sampling date, plants were dug out carefully. Since *V. album* roots are thick and white they could easily be distinguished from roots of other species. At times it proved impossible, however, to retrieve root tips when they disappeared into cracks in rocks. Root biomass is therefore slightly underestimated in the current study.

The entire dug-out plants were transported to the laboratory and stored overnight at 4°C. The next day, roots and rhizomes were washed; all plant parts were cut into c. 1 cm large pieces and dry weight was determined after 48 h at 80°C. Between excavation and drying was never > 24 h. We harvested and examined entire plants rather than sample only parts of them, as storage compounds are very heterogeneously distributed over organs and within organs over parts of different age. Nitrogen-based defence compounds (e.g. alkaloids) that may be produced in response to harvesting damage, are not produced in significant amounts until after 24 h (Ohnmeiss & Baldwin, 1994) and even then the concentrations are too low (Ohnmeiss & Baldwin, 2000) to confound the temporal patterns in N concentrations observed in different plant parts of *V. album*.

Before analysis of tissue N and carbohydrate content we pooled the biomass of the two harvested plants in each block.

This reduced the sample size from eight to four; however, it also reduced variation so that statistical power was not greatly reduced. Random samples of the pooled shoot, rhizome and root biomass were ground for analysis. Nitrogen content was determined using a CHN analyser (Model 932; LECO instruments, St Joseph, MI, USA). Starch and sugar (sucrose, glucose and fructose) were determined following the procedure described by Wong (1990) and Körner & Miglietta (1994). About 2 mg ground sample was boiled in distilled water for 30 min. One subsample of the solution was treated with invertase and isomerase and analysed spectrophotometrically for glucose using the sigma reagent (Hexokinase; Sigma, St Louis, MO, USA). Another subsample (including starch) was incubated with dialysed Clarase (fungal α -amylase of *Aspergillus oryzae*; Enzyme solutions Pty Ltd, Crydon south, Victoria, Australia). The filtrate was then treated as above and analysed for glucose. Each sample was analysed twice and two starch and glucose standards were used for each 96-well microtitre plate.

Vegetation and soil sampling

To determine growth of the surrounding, competing vegetation, on each sampling date after snowmelt, vegetation was cut at ground level in a radius of 25 cm from the shoot base of the control plants and dry weight was determined after 48 h at 80°C.

On each sampling date 10 soil cores (2.5 cm diameter, 10 cm deep) were taken in each block and pooled into a bulk sample. The samples were transported to the laboratory in a cool-box and stored overnight at 4°C. Time between collection and processing of the samples was never > 24 h. The samples were thoroughly mixed and fragments of rock and roots were removed by sieving using a 5 mm mesh. A subsample of 10 g was used to determine water content by drying for 48 h at 80°C. A fresh subsample of 20 g was suspended in 50 ml 1 M KCl, left shaking for 1 h, filtered and stored at -20°C until available N ($\text{NO}_3 + \text{NH}_4$) was analysed. Available N was measured spectrophotometrically using a segmented flow analyser (Skalar SAN^{plus} System; Skalar, Breda, the Netherlands).

Data analysis

Total N content of shoots, rhizomes and roots was obtained by multiplying the measured N concentration (in percentage dry weight) by the dry weight of the organs. Following Jaeger and Monson (1992), the quantity of stored N used to support shoot growth was calculated by adding the N content of the primordium before shoot expansion began (i.e. 10 March samples), to the decrease in N content of roots and rhizomes during the period of shoot growth (date of peak shoot biomass 7 June). In this period, N is presumably being translocated to the shoot and new root growth has not yet been initiated (D. Kleijn, pers. obs.) so that root and rhizome growth are no

major N sinks. Nitrogen uptake was determined as the maximum shoot N content minus the total amount of N derived from stores (primordium, plus root and rhizome depletion).

Early season growth initiation and growth rate of *V. album* was estimated by fitting logistic curves through all data collected in the year 2000 (GENSTAT, 1993):

$$DW_V = DW_i + (DW_{max}/1 + \exp(-b(x - m)))$$

(DW_V , *V. album* shoot dry weight (in g) at time x (in days since 1 January 2000); DW_i , initial dry weight before growth starts (g); DW_{max} , potential dry weight production (g); b , maximum relative growth rate (d^{-1}); m , day at which unrestricted growth changes to restricted growth). Vegetation growth was estimated similarly.

Statistical analysis

We analysed *V. album* responses to the treatments using ANOVA with treatment ('shoot removal', 'vegetation removal' and control; fixed factor) nested within blocks (random factor). As we were interested in differences between treatments rather than in changes through time we analysed the data of the two sampling dates independently. The analysis of the dry weight response was based on data from two replicate plants within each block ($n = 8$), whereas analyses examining the response of N and carbohydrate reserves was obtained from data of the two pooled plants in each block ($n = 4$). Blocking was primarily performed to reduce random environmental variation and thus to increase the statistical power of the analyses of treatment effects. Block effects are therefore not reported. Differences between means were tested for statistical significance using LSD-tests for planned comparisons (GENSTAT, 1993; Sokal & Rohlf, 1995) with a probability level (α) of 0.01 to compensate for

making multiple comparisons between means with each test. All biomass data were ln-transformed before analysis. All other data met assumptions of normality and homogeneity of variances required for analysis of variance (ANOVA).

Results

Temporal trends in N and carbohydrates

The snow cover disappeared from the study site between 11 and 18 April 2000. Shoot growth of *V. album* started immediately after snowmelt but did not commence earlier or more rapidly than that of the surrounding vegetation (Fig. 1).

Dry weight of all plant organs decreased from September to December 1999, remained fairly constant during the winter months, and increased markedly right after snowmelt (Fig. 2a). However, between 9 May and 7 June, in the middle of the period of most rapid shoot growth, rhizome and root dry weight dropped to a seasonal minimum. When shoot growth had stopped rhizome and root dry weight increased rapidly (Fig. 2a). The dry weight increase of the roots was at least partly due to the production of new roots that were formed in this period only.

Nitrogen concentration of shoots increased rapidly immediately following snowmelt, despite the increase in biomass, indicating that N transport to the shoot must have exceeded the diluting effect of growth. Halfway through the shoot growth period, shoot growth rate probably exceeded N supply by the rhizome and roots as shoot N concentration dropped considerably (Fig. 2). Nitrogen concentration of rhizomes and roots were relatively stable until the shoot reached maximum growth rate (between 9 May and 7 June 2000), after which N concentration declined considerably to remain low until the final sampling date at 19 July 2000 (Fig. 2b).

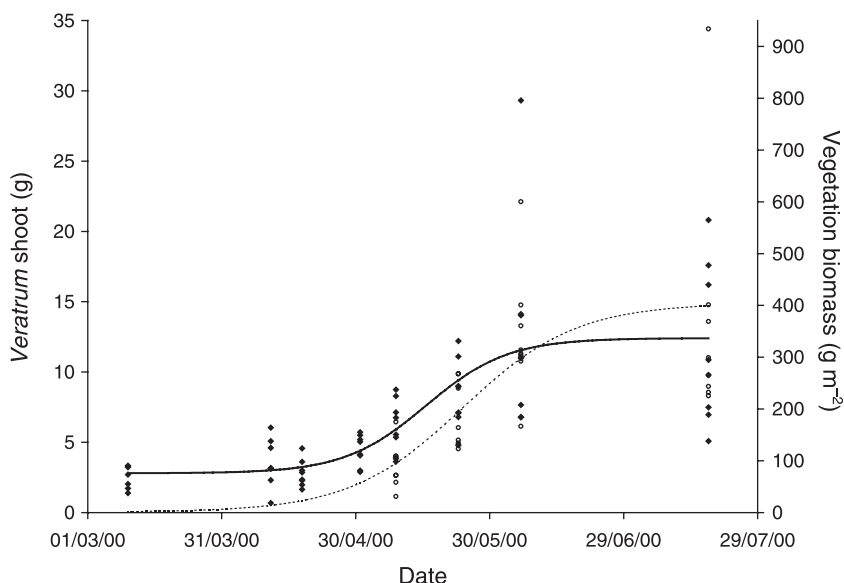


Fig. 1 *Veratrum album* shoot growth (diamonds, solid line) relative to the growth of the surrounding vegetation (circles, dashed line). Trend-lines were estimated by fitting logistic curves through all data collected in the year 2000 (GENSTAT, 1993). *Veratrum* shoot d. wt = $2.80 + (9.61/(1 + \exp(-0.11(\text{days since 1 January} - 136))))$; $R^2 = 51.5$; $F_{3,59} = 22.97$, $P < 0.001$. Vegetation d. wt = $1.04 + (403/(1 + \exp(-0.08(\text{days since 1 January} - 144))))$; $R^2 = 66.2$; $F_{3,60} = 42.05$, $P < 0.001$.

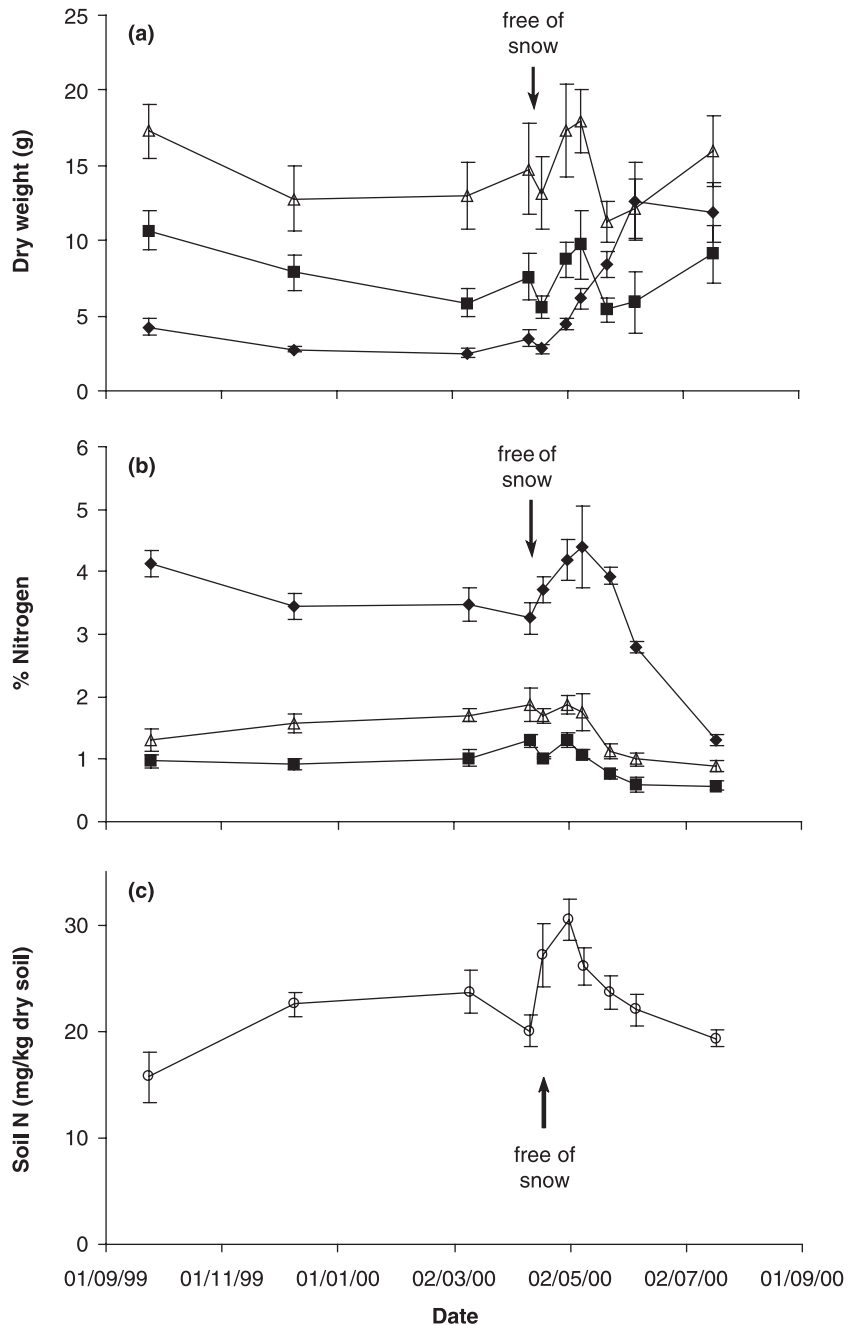


Fig. 2 Temporal trends in (a) dry weight and (b) nitrogen (N) concentration (% of dry weight) of *Veratrum alburn* shoots (diamonds), rhizomes (squares) and roots (triangles); (c) N availability ($\text{NO}_3 + \text{NH}_4$) in the soil. Symbols are means \pm SE, $n = 8$ for (a), and $n = 4$ (b,c).

In June and July shoot dry weight remained more or less constant whereas rhizome and root dry weight increased considerably (Fig. 2a). Concurrently, N concentration in shoots dropped dramatically while N concentration of rhizomes and roots remained stable (Fig. 2b), suggesting that a significant amount of N had been resorbed from shoot to rhizome and roots between 7 June and 19 July.

The temporal fluctuations in shoot N concentration corresponded closely with that of the availability of N in the soil (Fig. 2c). Soil available N showed a pronounced increase for c. 3 wk after snowmelt (between 18 April and 9 May), after

which it decreased to its original level. The increase in soil available N occurred at the same date as the increase in *V. alburn* shoot N concentration, while the subsequent decrease in soil N was observed 2 wk before the decrease in shoot N concentration (Fig. 2c).

Approximately 60% of the N used in the shoot at peak biomass originated from stored reserves, the remaining 40%, by definition, being derived from uptake (Fig. 3). Most N was stored in the roots and the shoot primordium, while the rhizome contributed relatively little to shoot N content.

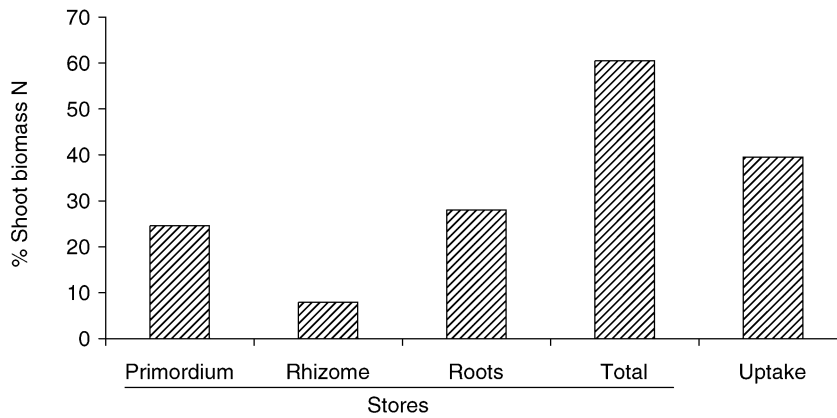


Fig. 3 Origin of nitrogen (N) invested in the shoot of *Veratrum album* during the growing season. The N content of *V. album* shoots at peak biomass (7 June 2000) was taken as 100%.

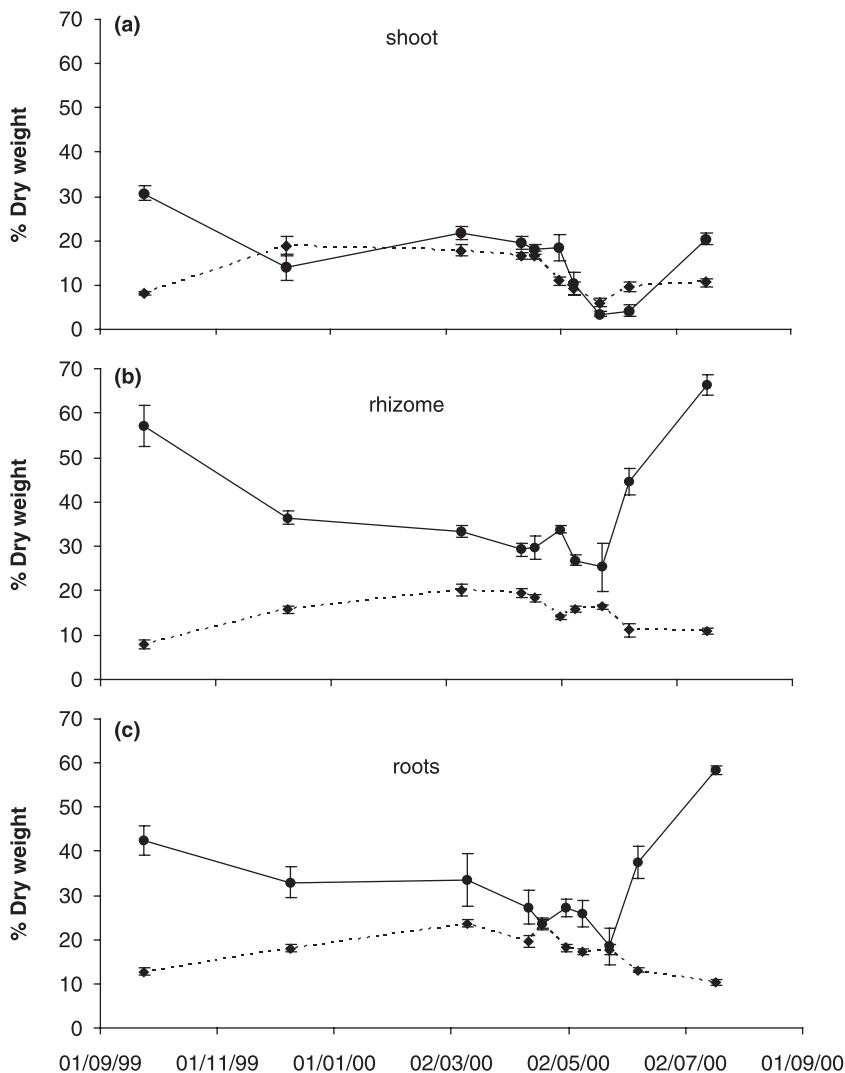


Fig. 4 Temporal trends in sugars (sucrose, glucose and fructose; diamonds, dashed line) and starch (circles, solid line) in (a) shoots (b) rhizomes and (c) roots of *Veratrum album*.

Sugar concentration showed similar temporal fluctuations in shoots, rhizomes and roots. It increased slightly during the dormant period in winter and decreased after snowmelt (Fig. 4). On average, sugar concentration was 12.4, 15.0 and 17.4% of organ dry weight for shoots, rhizomes and roots,

respectively. Starch concentration decreased in all plant parts between September and December 1999, but remained relatively constant from January to April. It then dropped considerably in May after which a rapid increase could be observed, particularly in the rhizomes and roots (Fig. 4b,c). The increase

Table 1 Results of the ANOVAS analysing treatment effects 2 wk and 8 wk after application on dry weight and stored reserves in shoots, rhizomes and roots of *Veratrum album*

	df _{treatment}	df _{error}	2 week		8 week	
			F	P	F	P
Dry weight						
Shoot ^a	1	11	0.22	0.649	0.00	0.964
Rhizome	2	18	0.31	0.740	4.12	0.034
Roots	2	18	0.39	0.685	9.57	0.001
Nitrogen (% d. wt)						
Shoot	2	6	10.92	0.010	11.22	0.009
Rhizome	2	6	1.21	0.361	20.37	0.002
Roots	2	6	1.17	0.372	14.63	0.005
Sugar (% d. wt)						
Shoot	2	6	1.35	0.329	5.71	0.041
Rhizome	2	6	1.32	0.335	0.45	0.659
Roots	2	6	2.41	0.171	0.20	0.825
Starch (% d. wt)						
Shoot	2	6	46.34	< 0.001	12.27	0.008
Rhizome	2	6	8.12	0.020	20.03	0.002
Roots	2	6	5.03	0.052	63.90	< 0.001

Differences between treatment means are given in Figs 5 and 6. For simplicity the effects of the random factor 'blocks' are not given.

^aAnalysis of shoot dry weight included only the treatments vegetation removal and control.

in starch concentration was somewhat later in shoots compared with rhizomes and roots. Mean starch concentration was highest in the rhizome (38.3% dry weight) followed by the roots (32.7%) and the shoot (16.1%).

Effects of shoot and vegetation removal on N and carbohydrates in *V. album*

The vegetation removal treatment did not result in *V. album* plants whose dry weight, N or carbohydrate concentrations deviated significantly from those in control plants (Table 1, Figs 5 and 6). By contrast, the removal of *V. album* shoots approx. 5 wk after emergence resulted in rhizome and root dry weights that were both only 43% of rhizome and root dry weights of the control plants (Table 1, Fig. 5). All plants that had the shoots removed survived until the end of the experiment, and by this time had developed a large primordium of next year's shoot.

Nitrogen concentration of the below-ground base of cut shoots was lower than that of control shoots 2 wk after, but significantly higher 8 wk after shoot removal when the remains of the original shoot had decayed but the primordium of the new shoot had grown to a considerable size. In rhizome and roots, N concentration of *V. album* plants with cut shoots was significantly higher than that in the control plants (Table 1, Fig. 5). Nevertheless, because biomass of shoot removal plants was much lower, at the final sampling date the N content (dry weight \times N concentration) was 33%

lower in shoot removal plants than in control plants (assuming a 50% resorption from the senescing shoots of the control plants; Eckstein *et al.*, 1999; Aerts & Chapin 2000).

On 19 July, N content (dry weight \times N concentration) of only the below-ground plant parts of shoot removal plants (including next year's shoot primordium) was similar to that of control plants (182.5 vs 195.1 mg N per plant, respectively). The N resorption that was observed in control plants between 7 June and 19 July (Fig. 2) had been made impossible in shoot removal plants, suggesting that shoot removal did not result in reduced or no N uptake.

Sugar concentration in rhizome and roots was not affected by above-ground removal of shoots (Table 1, Fig. 6). However, starch concentration was rapidly and drastically affected by shoot removal and starch concentration in rhizomes and roots was 48% and 44% of that in the control plants 8 wk after the treatment had been applied (Fig. 6). Starch concentration increased rapidly and reached significantly higher levels in the below-ground primordium of next year's shoot compared with the intact shoots of control plants (Table 1, Fig. 6). Starch content of shoot removal plants was 80% lower than that of control plants; a conservative estimate as the growing season had not yet completely ended at the final sampling date.

Discussion

Do reserves give *V. album* a competitive advantage?

Reserves do not give *V. album* a competitive edge early in the growing season. This conclusion is supported by three lines of argument. First, shoot growth of *V. album* and the surrounding vegetation started simultaneously, right after snowmelt. New roots were not produced until June and July (D. Kleijn, pers. obs.) so we can exclude the possibility that N kick-started early season below-ground growth. Second, *V. album* shoot N concentration did not increase before the increase in soil N availability but followed the temporal fluctuations of soil N availability closely. Early season growth supported by N reserves should have resulted in an increase in shoot N concentration before an increase in available soil N. Third, the vegetation removal treatment had no effect on *V. album* dry weight, N or carbohydrate content (Figs 5 and 6). This indicates that *V. album* growth is not limited by above-ground competition with the neighbouring vegetation, which makes an early season head-start in shoot growth supported by reserves unnecessary. In general, alpine plant growth does not appear to be particularly limited by carbon (Körner, 1999). Our results contrast with those of Chapin *et al.* (1986) who found that *Eriophorum vaginatum* reached its maximum growth rate early in the season supported entirely by N stored in the stem at a time when the roots were still frozen in the soil. The obvious difference is that *E. vaginatum* is an evergreen species that can use reserves to start growth when above-ground

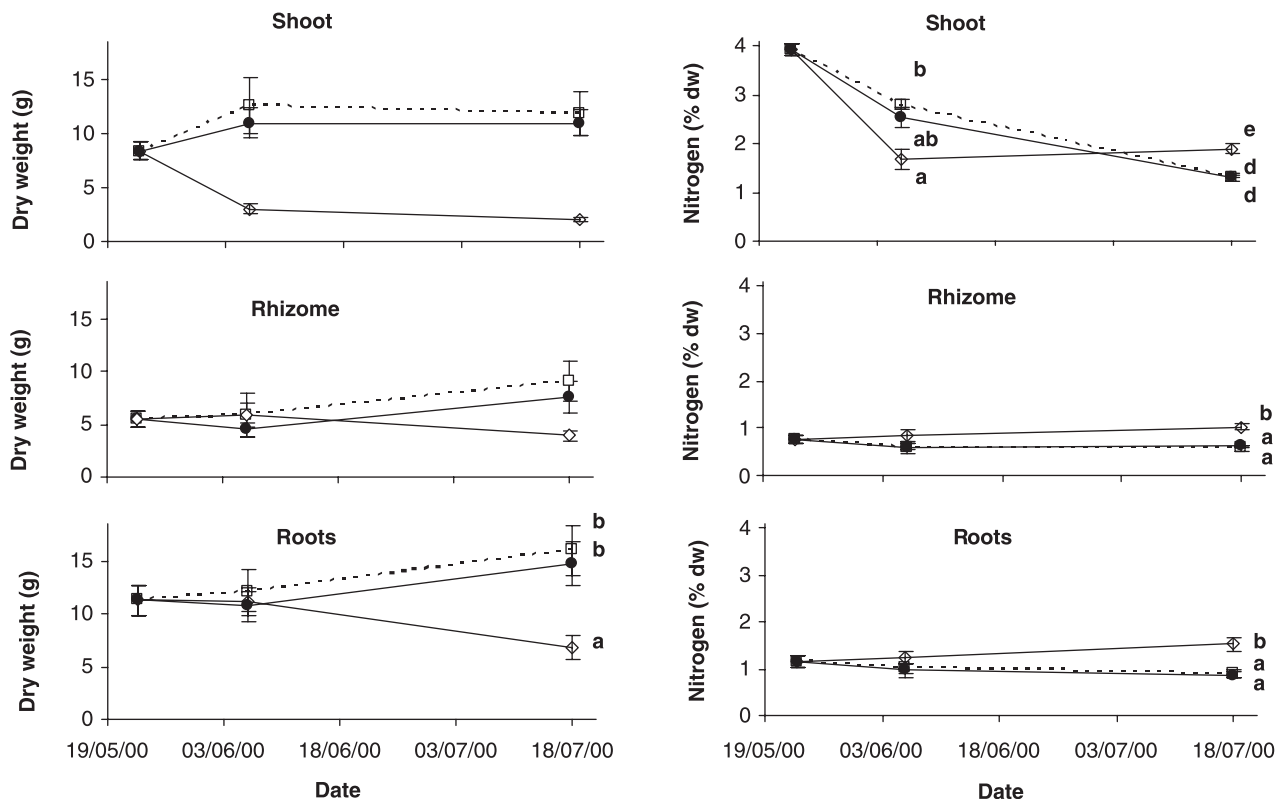


Fig. 5 The effects of vegetation removal (filled circles), shoot removal (open diamonds) and no interference (control, open squares, dashed line) on dry weight (left panels) and nitrogen (N) concentration (right panels) of *Veratrum album* shoots, rhizomes and roots. Treatments were applied on 23 May 2000. Shoot dry weight and N concentration of 'shoot removal plants' was measured in the below-ground shoot base and the developing primordium of next year's shoot. Shoot dry weight of 'shoot removal plants' was not included in the statistical analysis. Different letters indicate significant differences ($P < 0.01$) within sample dates only. Symbols indicate means \pm SE, $n = 8$ (dry weight) and $n = 4$ (N).

conditions are favourable, but below-ground conditions are not yet suitable for plant growth. For hemicryptophytic species such as *V. album*, below-ground conditions need to be favourable before above-ground growth can commence.

The period of most rapid shoot growth occurred during the period of maximum soil-N availability (Fig. 2). Nevertheless, shoot growth was supported for 60% by stored N. This is the same proportion as was found for the North-American hemicryptophytic alpine herb *Bistorta bistortoides* (Jaeger & Monson, 1992). Chapin *et al.* (1990) suggested plant growth is sustained by nutrient uptake from the soil in preference to stored reserves whenever possible but that plants depend substantially on stored reserves during periods of rapid growth. Jaeger & Monson (1992) proposed that the use of stored N in *Bistorta bistortoides*, which also occurred during periods of peak soil-N availability, was necessary to support the simultaneous development of both shoots and inflorescences made necessary by the short alpine growing season. *Veratrum album* rarely flowers and none of the plants sampled in this study had developed an inflorescence. The simultaneous transport of stored N and N taken up from the soil to the shoot resulted in a period of *c.* 1 month in which shoot N concentration was

approx. 4% and reached a peak level of 4.4%. This is more than twice the mean N concentration observed in forbs and graminoids (*c.* 1.6%; Aerts & Chapin, 2000).

The high N concentration may be related to the high levels of N-based alkaloids that render *V. album* unpalatable to all but a few specialist herbivores. Schaffner (1994) found that *V. album* shoot alkaloid concentration (measured as optical density) showed a similar, though less pronounced, decline through the growing season as the shoot N concentration. At present, it is not clear how much of the N present in the leaves should be considered 'defence N' (Aerts & Chapin, 2000) so that it is difficult to attribute the high shoot N concentration to a high concentration of alkaloids.

The seasonal N dynamics of *V. album* resembles the pattern observed in spring ephemerals, who, right after snowmelt, have a shoot N concentration of 4–5% which then rapidly declines as the growing season progresses (Muller, 1978; Anderson & Eickmeier, 2000). The carbon assimilation of a leaf is linearly related to the N content of that leaf (Hirose & Werger, 1987; Evans, 1989). For spring ephemerals it has been suggested that the observed high photosynthetic rate early in the season allows them to complete their above-ground

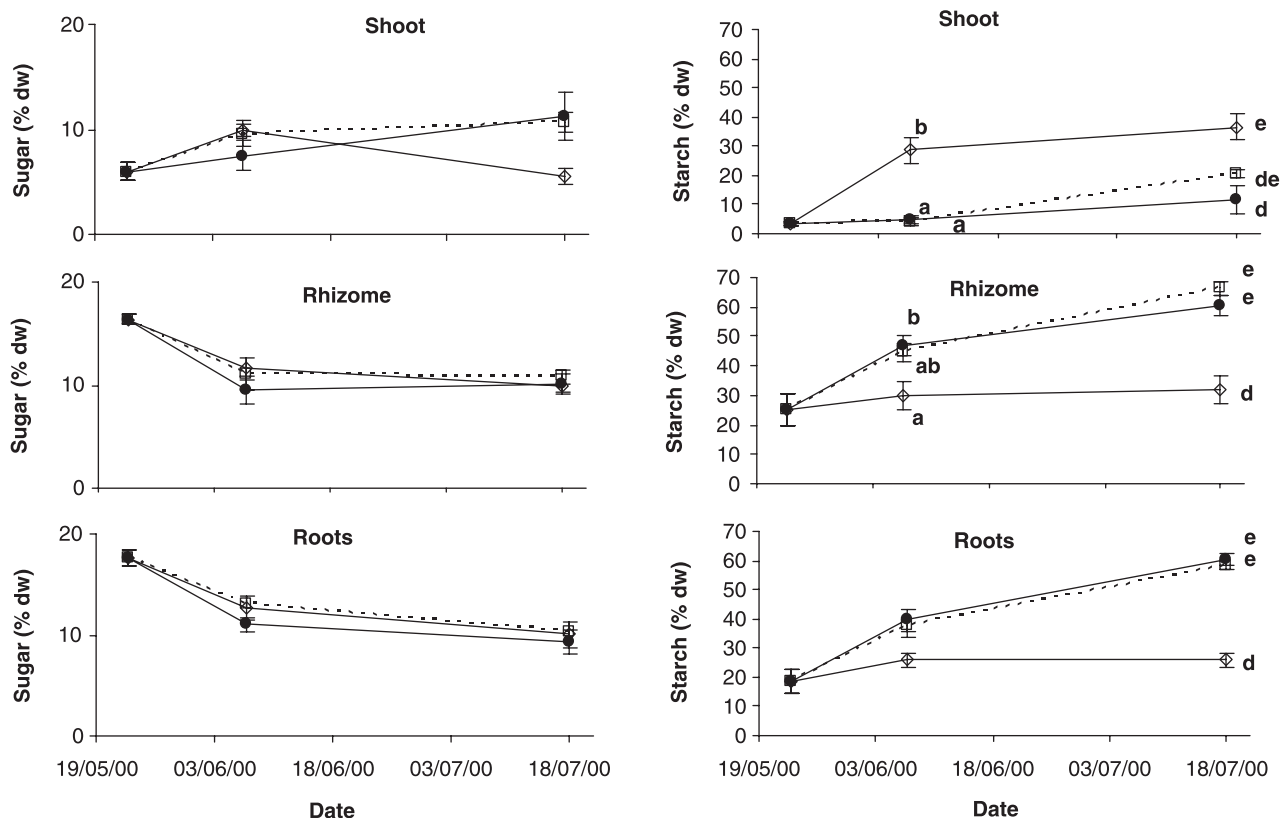


Fig. 6 The effects of vegetation removal (filled circles), shoot removal (open diamonds) and no interference (control; open squares, dashed line) on sugar concentration (left panels) and starch concentration (right panels) of *Veratrum album* shoots, rhizomes and roots. Treatments were applied on 23 May 2000. Sugar and starch concentration of 'shoot removal plants' was measured in the below-ground shoot base and the developing primordium of next years shoot. Different letters indicate significant differences ($P < 0.01$) within sample dates only. Symbols indicate means \pm SE, $n = 4$.

growth cycle in the short time span before the forest canopy closes (Lapointe, 2001). Similarly, the early season high nutrient concentrations may allow *V. album* to refill the carbohydrate stores used up during autumn, winter and early spring growth in a short period of time. Such a strategy would allow *V. album* to senesce well before the growing season ends so that early season frosts are avoided and the maximum possible amount of nutrients can be resorbed from the senescing shoot. This is corroborated by the seasonal carbohydrates dynamics (Fig. 4).

The decline in starch concentration in autumn was considerable, possibly owing to the relatively high temperatures and the associated high respiration rates (Ögren, 1997; Bruelheide & Lieberum, 2001). Following a relatively stable period in winter, both sugar and starch concentrations declined briefly in spring during the period of most active shoot growth. The modest impact of shoot growth on the carbohydrate reserves suggest that, although initially growth is entirely dependent upon reserves (Ho & Rees, 1975), after the leaves have emerged, much of the carbohydrates needed for shoot growth are assimilated by the developing shoot itself (Ho & Rees, 1977; van der Heyden & Stock, 1996). Once the shoot was

fully grown and no longer acted as a sink for assimilates, starch concentration in roots and rhizome increased rapidly. An important function of the N reserves in *V. album* may therefore be risk aversion as it allows the plant to complete its growing season in the shortest time possible.

Do reserves help *V. album* survive calamities?

As intended at the outset of the study, our shoot removal treatment was applied at a time when carbohydrate reserves in below-ground organs were at a minimum. Shoot N concentration and N content were close to maximal when the shoot removal treatment was applied (89% and 94% of maximal, respectively). As cut *V. album* shoots do not regrow in the same growing season, the shoot removal treatment represented the worst possible defoliation calamity. The impacts of the treatment were severe, particularly on the carbohydrate reserves. At the final sampling date, N content of the shoot removal plants was two-thirds of that of control plants, while starch content of shoot removal plants was only 20% of that of control plants. Zimmerman and Whigham (1992) studied the effects of complete defoliation on the

carbohydrate reserves of the orchid *Tipularia discolor*. This species maintains extensive below-ground reserves and produces a single leaf each year. Like *V. album*, when the leaf is lost during the growing season, it does not resume growth until the next growing season. Defoliation resulted in a reduction of nonstructural carbohydrates of approx. 65%.

The difference in impact of shoot removal on N and carbohydrate reserves may be explained by the fact that, at the time of the removal of the shoot, much of the peak in soil N availability had already passed and most N had been taken up. Furthermore, our results indicate that N uptake continues even after removal of the entire shoot. By contrast, at the time of shoot removal, the amount of below-ground carbohydrate reserves is minimal. In the subsequent period, in June and July, the control plants have a high rate of carbohydrate assimilation and most carbohydrates are transported to the roots and rhizomes, whereas the shoot removal plants did not produce any assimilates.

Despite the strong impact of the shoot removal treatment on the carbohydrate reserves in particular, a considerable amount of carbohydrates (on average 3.0 g per plant) remained available to the plants. We did not observe any mortality among the shoot removal plants. Schaffner *et al.* (1995) examined the response of *V. album* plants to seven consecutive years of mowing. In the seventh year, the mowing treatment had reduced mean plant dry weight by 50% but had a negligible effect on plant mortality. The dry weight of below-ground organs of their plants was comparable with that observed in the shoot removal plants in our study (9.5 g and 10.7 g per plant, respectively). This suggests that although calamities significantly reduce reserves of N and especially carbohydrates, the remaining reserves appear to be more than sufficient to survive until the next growing season. The modest (compared with the present study) effect of the mowing treatment of Schaffner *et al.* (1995) probably resulted from the time of mowing, which was executed when reserves had already been refilled to a considerable extent.

The ecological significance of reserves in *V. album*

Rather than giving *V. album* a competitive edge in early spring and/or being an insurance against calamities, the integral function of both N and carbohydrate reserves appears to be risk avoidance. Reserves allow *V. album* to complete the above-ground growing cycle in as short a period as possible, thus minimizing exposure to above-ground risks such as trampling or early frosts in late summer or autumn. The N reserves are used to supplement N uptake so that high leaf N concentrations and assimilation rates can be maintained and depleted carbohydrate reserves can be refilled rapidly. In addition, an unknown proportion of plant N is used to defend the plant against herbivores so that little of the assimilative apparatus is lost during the short growing season. The carbohydrate reserves are subsequently used to survive

the dormant period that lasts three-quarters of the year and may also be used to buffer the impact of any calamity that prevents the plant from refilling its reserves during the growing season.

The physiological adaptations facilitated by stored reserves allow *V. album*, a species with very low phenotypic plasticity (e.g. a single preformed shoot per year and unresponsive root system) to persist in habitats with a short and often unpredictable growing season and compete successfully with plants demonstrating high phenotypic plasticity and few reserves. Further research is merited to examine whether species with similar phenotypic constraints (e.g. many species of orchids and spring ephemerals) have similar reserve dynamics.

Acknowledgements

We kindly thank O. Niederberger for allowing us to work on his pasture. The advice and help of C. Körner, G. Hoch and O. Bignucolo and the assistance of M. Kleijn, I. Rem, M. Käser and J. van Walsum in the field and laboratory is much appreciated. This work was partly funded by the Swiss National Science Foundation through the National Centre of Competence in Research (NCCR) Plant Survival, and the National Research Programme 48 on Landscapes and Habitats of the Alps.

References

- Aerts R, Chapin FS III. 2000. The mineral nutrition of wild plants revisited: a re-evaluation of processes and patterns. *Advances in Ecological Research* 30: 1–67.
- Anderson WB, Eickmeier G. 2000. Nutrient resorption in *Claytonia virginica* L. implications for the deciduous forest nutrient cycling. *Canadian Journal of Botany* 78: 832–839.
- Bloom AJ, Chapin FS III, Mooney HA. 1985. Resource limitation in plants – an economic analogy. *Annual Review of Ecology and Systematics* 16: 363–392.
- Boyce PJ, Volenec JJ. 1992. Taproot carbohydrate concentrations and stress tolerance of contrasting alfalfa genotypes. *Crop Science* 32: 757–761.
- Bruehlheide H, Lieberum K. 2001. Experimental test for determining the causes of the altitudinal distribution of *Meum athamanticum* Jacq. in the Harz Mountains. *Flora* 196: 227–241.
- Chapin FS III, Shaver GR, Kedrowski RA. 1986. Environmental controls over carbon, nitrogen and phosphorus fractions in *Eriophorum vaginatum* in Alaskan tussock tundra. *Journal of Ecology* 74: 167–195.
- Chapin FS III, Schulze ED, Mooney HA. 1990. The ecology and economics of storage in plants. *Annual Review of Ecology and Systematics* 21: 423–447.
- Eckstein RL, Karlsson PS, Weih M. 1999. Leaf life span and nutrient resorption as determinants of plant nutrient conservation in temperate-arctic regions. *New Phytologist* 143: 177–189.
- Evans JR. 1989. Photosynthesis and nitrogen relationships in leaves of C₃ plants. *Oecologia* 78: 9–19.
- Fitter AH. 1987. An architectural approach to the comparative ecology of plant root systems. *New Phytologist* 106: 61–77.
- GENSTAT 5 Committee of the Statistics Department. 1993. *Genstat 5 release 3 reference manual*. Oxford, UK: Oxford University Press.
- Heilmeyer H, Schulze ED, Whale DM. 1986. Carbon and nitrogen partitioning in the biennial monocarp *Arctium tomentosum* Mill. *Oecologia* 70: 466–474.

- Hirose T, Werger MJA. 1987. Nitrogen use efficiency in instantaneous and daily photosynthesis of leaves in the canopy of a *Solidago altissima* stand. *Physiologia Plantarum* 70: 215–222.
- Ho LC, Rees AR. 1975. Aspects of translocation of carbon in the tulip. *New Phytologist* 74: 421–428.
- Ho LC, Rees AR. 1977. The contribution of current photosynthesis to growth and development in the tulip during flowering. *New Phytologist* 78: 65–70.
- Jaeger CH, Monson RK. 1992. Adaptive significance of nitrogen storage in *Bistorta bistortoides*, an alpine herb. *Oecologia* 92: 578–585.
- Kleijn D, Steinger T. 2002. Contrasting effects of grazing and hay-cutting on the spatial and genetic population structure of *Veratrum album*, an unpalatable, long-lived, clonal plant species. *Journal of Ecology* 90: 360–370.
- Kobe RK. 1997. Carbohydrate allocation to storage as a basis of interspecific variation in sapling survivorship and growth. *Oikos* 80: 226–233.
- Körner C. 1999. *Alpine plant life: functional plant ecology of high mountain ecosystems*. Berlin Heidelberg, Germany: Springer-Verlag.
- Körner C, Miglietta F. 1994. Long-term effects of naturally elevated CO₂ on Mediterranean grassland and forest trees. *Oecologia* 99: 343–351.
- Körner C, Renhardt U. 1987. Dry matter partitioning and root length/leaf area ratios in herbaceous perennial plants with diverse altitudinal distribution. *Oecologia* 74: 411–418.
- Lapointe L. 2001. How phenology influences physiology in deciduous forest spring ephemerals. *Physiologia Plantarum* 113: 151–157.
- Lipson DA, Bowman WD, Monson RK. 1996. Luxury uptake and storage of nitrogen in the rhizomatous alpine herb, *Bistorta bistortoides*. *Ecology* 77: 1277–1285.
- Lipson DA, Schmidt SK, Monson RK. 1999. Links between microbial population dynamics and nitrogen availability in an alpine ecosystem. *Ecology* 80: 1623–1631.
- Muller RN. 1978. The phenology, growth and ecosystem dynamics of *Erythronium americanum* in the northern hardwood forest. *Ecological Monographs* 48: 1–20.
- Ögren E. 1997. Relationship between temperature, respiratory loss of sugar and premature dehardening in dormant Scots pine seedlings. *Tree Physiology* 17: 47–51.
- Ohnmeiss TE, Baldwin IT. 1994. The allometry of nitrogen allocation to growth and inducible defense under nitrogen limited growth. *Ecology* 75: 995–1002.
- Ohnmeiss TE, Baldwin IT. 2000. Optimal defense theory predicts the ontogeny of an induced nicotine defense. *Ecology* 81: 1765–1783.
- Schaffner U. 1994. Interactions between *Veratrum album* L. and its herbivores: prospects of biology control of this native weed. PhD Thesis. Bern, Switzerland: University of Bern.
- Schaffner U, Kleijn D, Brown V, Müller-Schärer H. 2001. *Veratrum album* L. in montane grasslands: a model system for implementing biological control in land management practices of high biodiversity habitats. *Biocontrol News and Information* 22: 19N–28N.
- Schaffner U, Nentwig W, Brändle R. 1995. Effect of mowing, rust infection and seed production upon C and N reserves and morphology of the perennial *Veratrum album* L. (Liliales, Melanthiaceae). *Botanica Helvetica* 105: 17–23.
- Sokal RR, Rohlf FJ. 1995. *Biometry*. New York, NY, USA: Freeman.
- van der Heyden F, Stock WD. 1996. Regrowth of a semiarid shrub following simulated browsing: the role of reserve carbon. *Functional Ecology* 10: 647–653.
- Wong SC. 1990. Elevated atmospheric partial pressure of CO₂ and plant growth. II. Non-structural carbohydrate content in cotton plants and its effect on growth parameters. *Photosynthesis Research* 23: 171–180.
- Wyka T. 1999. Carbohydrate storage and use in an alpine population of the perennial herb, *Oxytropis sericea*. *Oecologia* 120: 198–208.
- Zimmermann JK, Whigham DF. 1992. Ecological functions of carbohydrates stored in corms of *Tipularia discolor* (Orchidaceae). *Functional Ecology* 6: 575–581.