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The effect of reproduction on nitrogen use-efficiency of three species of the carnivorous genus *Pinguicula*

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Summary

1 Life history theory predicts that reproduction incurs costs in terms of future growth and survival. In infertile environments reproductive events may accelerate nutrient turnover such that losses of limiting nutrients cannot be replenished by uptake from the soil.

2 We compared the effect of reproduction on nitrogen (N) turnover and N useefficiency, i.e. the annual dry matter produced per unit N lost, of three carnivorous species of the genus *Pinguicula* on an infertile subarctic heath. We hypothesized that reproduction should increase N turnover, unless a larger N pool or improved N resorption can compensate for the losses associated with reproduction.

3 These carnivorous herbs showed a nitrogen use strategy similar to that of noncarnivorous plants (herbs and grasses) with a relatively low mean residence time (MRT) of N and a large dry matter productivity per unit N in the plant (annual N productivity). N pool size and the efficiency of N resorption from senescent leaves were similar in reproductive and non-reproductive individuals. Reproductive individuals had significantly larger annual N losses and thus a lower average MRT (0.8 vs. 1.8 years), with values < 1 indicating that the annual N losses are greater than the annual average N pool. Unless plants can enhance their nutrient acquisition, flowering may therefore impact on future growth and survival.

4 This study presents evidence for a direct link between reproduction, reflected in a higher relative allocation to inflorescences, turnover and N use-efficiency; the latter being reduced because of increased N turnover. The ranking of species in terms of reproductive allocation was the reverse of their ranking in terms of their N use-efficiency.

Key-words: carnivorous plants, costs of reproduction, life-history, mean residence time, nitrogen productivity, nitrogen use-efficiency, *Pinguicula*, plant strategies, reproductive allocation, subarctic.

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Introduction

On chronically infertile soils nutrient acquisition is not limited by uptake kinetics of the roots but by the slow diffusion of ions in the soil solution (Nye 1977; Chapin 1980). Although plants of such environments often show specific adaptations to improve nutrient acquisition (Ernst 1983; Pate 1994), they are also characterized by a suite of traits that help to reduce nutrient

†Present address and correspondence: R. Lutz Eckstein, Department of Landscape Ecology, Justus-Liebig-University Gießen, Heinrich-Buff-Ring 26–32, DE-35392 Gießen, Germany (tel. +49 (0)641-99 37 188; fax +49 (0)641-99 38 169; email Lutz.Eckstein@agrar.uni-giessen.de). losses from the plant (Berendse & Aerts 1987; Aerts 1990, 1995, 1999; Chapin *et al.* 1993; Eckstein & Karlsson 1997; Eckstein *et al.* 1999; Aerts & Chapin 2000), such as slow growth, low tissue nutrient concentrations and low tissue turnover.

Carnivory is one adaptation to enhance nutrient acquisition, that is utilized by a small set of plant species of infertile habitats (Givnish 1989). The benefits of carnivory (see review by Adamec 1997) include increased growth, higher flowering frequency, seed mass and nutrient concentrations (Aldenius *et al.* 1983; Karlsson *et al.* 1991; Thorén & Karlsson 1998) and increased survival (Zamora *et al.* 1997) in plants that succeed in catching prey. Furthermore, it has been suggested that prey capture may increase soil nutrient

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uptake in carnivorous plants (Aldenius *et al.* 1983; Hanslin & Karlsson 1996; Karlsson *et al.* 1996). In species of the genus *Pinguicula*, the nitrogen (N) gain through prey capture has been estimated to range between 15% and 40% and between 7% and 26% of the annual N loss of non-reproductive and reproductive plants, respectively (Karlsson *et al.* 1994), thus reducing the amount of N that has to be taken up from the soil. However, the high variation of seasonal catches among individuals and years (Karlsson *et al.* 1994) renders carnivory an unreliable source of nutrient income.

On the other hand, carnivory in plants is probably also associated with considerable costs (Benzing 1987), for instance when leaves are used for both carbon assimilation and prey capture. This may, for example, lead to reduced photosynthetic production per unit leaf nitrogen when carnivorous *Pinguicula* species are compared with non-carnivorous species in similar environments (Méndez & Karlsson 1999). Such costs might be the cause of the relative rarity of carnivory, at only about 0.2% of all vascular plants, i.e. 500–600 species (Givnish 1989). The low competitive ability of the mostly small carnivorous plants further suggests that they should be restricted to habitats where water and light are abundant but nutrient availability is low (Givnish *et al.* 1984; Karlsson *et al.* 1991, 1996).

Although aspects of the resource economy of carnivorous plants have been studied (e.g. Karlsson 1988; Thum 1988; Karlsson et al. 1991; Thorén 1998) none, to our knowledge, has directly related their performance (in terms of productivity) to the flux of growthlimiting nutrients (uptake or loss). Applying the concept of nutrient use-efficiency (NUE) (Berendse & Aerts 1987; Garnier & Aronson 1998) to carnivorous perennials could shed further light on the consequences of carnivory and reproduction on plant nutrient dynamics. The separation of NUE into the productivity per unit nutrient (annual nutrient productivity, aNP) and the mean residence time of nutrients (MRT) as defined in the concept of Berendse & Aerts (1987) allows for a functional interpretation of NUE in terms of different nutrient use strategies (Eckstein & Karlsson 1997; Berendse 1998).

The principle of allocation (Willson 1983; Silvertown & Lovett Doust 1993) is based on the assumption that different plant functions compete for a limited resource pool. Reproduction should therefore incur a cost that is expressed as negative consequences either on growth (somatic costs) or future survival and fecundity (reproductive costs, cf. Stearns 1992). In general, empirical studies on costs of reproduction have not found consistent results (e.g. Reekie & Bazzaz 1987; Horvitz & Schemske 1988; Obeso 1993; Syrjänen & Lehtilä 1993). For the genus Pinguicula, however, significant costs of reproduction have been identified in both somatic (Thorén et al. 1996) and demographic terms (Svensson et al. 1993), with survival probabilities significantly reduced in reproductive individuals. In infertile environments a cost of reproduction could

© 2001 British Ecological Society, *Journal of Ecology*, **89**, 798–806 be mediated by flower and seed production accelerating nutrient turnover, leading to increased nutrient losses that cannot be replenished by uptake from the soil.

To test for the proposed link between reproduction and nutrient turnover we studied the effect of reproductive status on N use-efficiency (NUE) of three species of Pinguicula - P. alpina L., P. villosa L. and P. vulgaris L. - under field conditions. The reproductive allocation in terms of N, i.e. the proportion of the total N pool allocated to reproductive structures, is on average 0.3, 0.6 and 0.4 for P. alpina, P. villosa and P. vulgaris, respectively (Karlsson 1988; Karlsson et al. 1990; Thorén et al. 1996; Hemborg & Karlsson 1998). Although reproductive plants usually have larger biomass and nutrient pools than vegetative plants during the growing season, they also lose more nutrients through senescing reproductive structures in the autumn (Karlsson 1986, 1988; Thorén et al. 1996). Winterbuds are therefore usually smaller and contain less N after flowering than those of non-reproductive plants. This fact is expressed as a relative somatic cost of reproduction (RSC, Thorén et al. 1996).

The mean residence time of nutrients (MRT) is defined as the ratio between the average nutrient pool and the annual nutrient losses, and nutrient productivity (aNP) is the ratio between annual production and the average nutrient pool (Berendse & Aerts 1987; Aerts 1990; Garnier & Aronson 1998). Since both components of NUE are ratios, it is not obvious how reproduction will affect MRT and aNP but we hypothesize that it may lead to a proportionally larger increase in N losses than in average N pool size. Increased N turnover (lower MRT) in reproductive, plants would lead to a higher annual N requirement and thus explain the negative effects of flowering on future fate unless this can be compensated for by soil- or prey-derived nutrients. We test whether N use-efficiency of reproductive plants is indeed lower than in non-reproductive individuals.

Methods

THE SPECIES

Three species of the genus *Pinguicula* (Lentibulariaceae) are represented in the Swedish subarctic: *P. alpina, P. villosa* and *P. vulgaris*. All three are iteroparous herbaceous geophytes with a basal leaf rosette, relatively few unbranched roots, and one or a few flower stalks, each bearing a single flower. The perennating organ is a winter bud that develops in late summer and is situated below the soil surface during the unfavourable season for growth. In the study area, *P. alpina* mainly grows on frost-heaved, calcareous soils on solifluction terraces or polygons (Karlsson 1986; Svensson *et al.* 1993). This early flowering species has a perennial root system. *P. villosa* is the smallest Swedish *Pinguicula* species and occurs as epiphyte on nutrient-poor 800 R. L. Eckstein & P. S. Karlsson hummocks of *Sphagnum fuscum* (Schimp.) Klinggr. (Svensson *et al.* 1993; Thorén 1998). *P. vulgaris* has the broadest distribution of these three species and occurs in a range of habitats from calcareous to acidic soils. It prefers more stable soils (Karlsson 1986; Svensson *et al.* 1993; Thorén 1998) and flowers later than *P. alpina* (Molau 1993).

STUDY SITES

The study was carried out in the vicinity of the Abisko Scientific Research Station ($68^{\circ}21'$ N, $18^{\circ}49'$ E, c. 360 m elevation) in northernmost Sweden. The studied populations were situated close to permanent plots described in detail by Karlsson *et al.* (1991) and Svensson *et al.* (1993). Each species was studied in the habitat where it is most commonly found, i.e. active polygons (*P. alpina*), stabilized polygons (*P. vulgaris*) and *Sphagnum fuscum* hummocks (*P. villosa*). Specimens were sampled from several locations spread over a larger area (c. 100 by 100 m) to include some of the spatial heterogeneity of the habitat.

HARVESTING PROCEDURE

In early June 1996, 120 individuals of each species and reproductive status (reproductive/non-reproductive) were tagged and randomly assigned to one of six harvests made at about 3-week intervals during the growing season, to represent the following developmental stages: (i) flower buds visible, (ii) flower stalk developed, (iii) and (iv) around peak biomass and flowering, (v) winter bud developed, and (vi) overwintered buds collected in early June 1997, immediately after snow melt. In June 1997, a further 60 plants of each species and status were tagged and randomly assigned to harvest (vii), (viii) or (ix), corresponding to harvests (i), (iii) and (v) in 1996.

At each harvest, the plants were excavated with their surrounding substrate in the field and immediately brought to the laboratory. Here they were carefully cleaned and separated into live and senescent tissues and into leaves, roots, budscales, winter buds and reproductive structures to include both flower and flower stalk. All plant parts were dried at 70 °C for 24 h and weighed to the nearest 0.1 mg.

CHEMICAL ANALYSES AND CALCULATIONS

Total Kjeldahl nitrogen (N) was analysed after digestion in sulphuric acid using a flow injection analyser (Fia-star 5012 system, Foss Tecator AB, Höganäs, Sweden). We ignored nitrate-N because nitrate is present, if at all, in very low amounts in nutrient-poor heath systems of the study area (Jonasson *et al.* 1993; Weih 1998). In *P. alpina* and *P. vulgaris*, we analysed the pooled living vegetative parts (i.e. leaves + roots + winter bud) of individual plants and the reproductive structures (i.e. flower and flower stalk) separately (n = 10 for each species, reproductive status and harvest combination). Due to low dry mass and N content, senescent tissues had to be analysed as pooled samples of five (reproductive structures) to 20 plants (leaves and budscales). In *P. villosa*, five plants had to be pooled to get enough vegetative parts or reproductive structures for the chemical analysis. We specifically refer to N in this study as this is the major growth-limiting mineral nutrient in lowland mire and heath ecosystems of the study area (Aerts *et al.* 1992; Jonasson *et al.* 1993).

N pool sizes of live and dead vegetative parts and inflorescences were calculated using the mean N concentration and the mean dry mass of the appropriate plant fractions. The average annual N pool in 1996 and 1997 was estimated as a weighted average over the whole year. We used an estimated N pool during the periods not represented by harvests. For the winter period 1996–97, we used the mean N pool of harvests (v) and (vi), for the remaining part of 1997 we used the winterbud N pool of the harvest (ix), and for the time period prior to the first harvest in 1996 we averaged the N pool of harvests (v) (vi) and (ix).

From the harvest with the maximum number of green leaves per plant, we analysed an additional five (pooled) samples of green leaves per species and reproductive status. Relative resorption of N during leaf senescence (R_{EFF}) was then estimated by relating the difference in N pools per leaf between green and senescent leaves to the nutrient pools of green leaves. Furthermore we estimated the leaf resorption proficiency (R_{PROF}), i.e. the level to which N concentrations were reduced in senescent leaves (Killingbeck 1996).

N losses from the plants were estimated from seasonal changes of N pool size of different plant parts and comprise the following fractions:

• The difference in N pool of vegetative plant parts between maximum N pool and autumn N pool. These losses include shed leaves and roots as well as losses due to leaching.

• Budscales and leaves senescing before maximum N pool size was reached. The scales protecting the winterbud were distinguished from leaves by their small size and lack of glands and were weighed separately from leaves.

• Losses due to the shedding of seeds were estimated indirectly from the difference in N pool of reproductive structures, i.e. capsule plus stalk, between peak season and autumn. These losses include shed seeds as well as possible resorption from the stalks and leaching losses from the senescent capsules and stalks.

• The amount of N found in senescent empty reproductive structures in autumn.

• Freshly shed petals were collected in populations of all three species and weighed and analysed as pooled samples for each species and year.

Dry matter productivity was estimated as the increase in dry matter between plants in the winterbud stage and the maximum standing crop. The study started relatively late in 1996 as we wanted to make sure

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Table 1 Components of the annual nitrogen loss (μ mol N plant⁻¹ year⁻¹) of non-reproductive (NR) and reproductive (R) individuals of three subarctic *Pinguicula* species during 2 years (1996, 1997). Figures in brackets give the percentage contribution of different loss fractions

	1996		1997	
	NR	R	NR	R
Pinguicula alpina				
Annual loss through:				
Budscales + early leaves	0.0 (0.0)	0.0 (0.0)	0.8 (17.0)	0.9 (8.8)
Leaves + roots	2.2 (100.0)	2.1 (35.0)	3.9 (83.0)	4.7 (46.1)
Petal	_	1.0 (16.7)		0.9 (8.8)
Seeds	-	2.3 (38.3)	-	3.0 (29.4)
Capsule + stalk	-	0.6 (10.0)	-	0.7 (6.9)
Total	2.2	6.0	4.7	10.2
Pinguicula villosa				
Annual loss through:				
Budscales + early leaves	0.3 (13.6)	0.3 (6.8)	0.9 (64.3)	0.0 (0.0)
Leaves + roots	2.2 (86.4)	1.1 (25.0)	0.5 (35.7)	2.2 (37.3)
Petal	-	0.2 (4.5)	-	0.2 (3.3)
Seeds	-	1.8 (40.9)	-	2.5 (42.4)
Capsule + stalk	-	1.0 (22.7)	-	1.0 (16.9)
Total	2.5	4.4	1.4	5.9
Pinguicula vulgaris				
Annual loss through:				
Budscales + early leaves	1.8 (25.0)	0.0 (0.0)	1.6 (32.0)	0.0 (0.0)
Leaves + roots	5.4 (75.0)	4.7 (39.5)	5.0 (68.0)	6.4 (48.9)
Petal	-	0.5 (4.2)	-	0.5 (3.8)
Seeds	_	5.6 (47.1)	_	4.5 (34.4)
Capsule + stalk	_	1.1 (9.2)	-	1.7 (13.0)
Total	7.2	11.9	5.0	13.1

that reproductive and vegetative plants could be clearly identified. Therefore, the initial dry mass in 1996 was assumed to be the average dry mass of harvests (v) (vi) and (ix).

The mean residence time (MRT) of N and annual N productivity (aNP) were calculated as the ratio between average N pool and annual N losses and as the ratio between dry matter productivity and average N pool, respectively (cf. Berendse & Aerts 1987). N use-efficiency (NUE) is then simply the product of MRT and aNP.

STATISTICS

The effects of species (d.f. = 2) and reproductive status (reproductive vs. non-reproductive, d.f. = 1) on various variables related to plant productivity and N use were tested using a fixed factor two-way analysis of variance with the two study years as replicates. Since the tested variables were based on means using data from the different harvests, sample sizes were small (n = 12). Therefore, we employed a permutation ANOVA procedure (Edgington 1987; Manly 1991). The permutations were based on an 'approximate randomization' procedure (Noreen 1989) and 5000 permutations were calculated as suggested by Potvin & Roff (1993). The error probability (P-value) in permutation analysis is the proportion of the permutations resulting in an F-value equal to or larger than the original F-value.

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Results

COMPONENTS OF THE ANNUAL N LOSS

The largest absolute N losses per plant and year were found in *P. vulgaris* and *P. alpina*, with the smallest species, *P. villosa*, showing the lowest annual N loss (Table 1). The seasonal course of the N pool for *P. alpina* differed between years (Fig. 1), with a clear peak during July 1997, especially in reproductive plants, while there was no distinct peak in 1996 (Fig. 1a). In contrast, *P. villosa* and *P. vulgaris* showed relatively similar patterns during the 2 years of the study (Fig. 1b,c), characterized by a distinct peak in August (1996) or July (1997).

Reproductive plants of all species showed significantly higher annual N losses than non-reproductive individuals (Table 1). In the latter, all losses were due to shed leaves and roots and possible leaching losses. Across all species and years, significant losses (mean 14%, range 0-64%) occurred before the maximum N pool size was reached but this proportion depended on the seasonal course of the N pool.

On average 41% (range 32-55%) of the annual N losses in reproductive plants occurred through vegetative parts, i.e. leaves, roots and budscales. The proportion of N lost as seeds was highest in *P. villosa* (42%) followed by *P. vulgaris* (41%) and *P. alpina* (34%) (Table 1), with *P. villosa* also showing the highest



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Fig. 1 Seasonal course of nitrogen (N) pool size (μ mol N plant⁻¹) of non-reproductive and reproductive individuals of *Pinguicula alpina* (a), *P. villosa* (b) and *P. vulgaris* (c) during 2 years (1996, 1997). Hatched = live vegetative plant fractions (leaves, roots and budscales); crosshatched = live reproductive structures (capsule, stalk); black = senescent leaves and budscales; white = senescent reproductive structures.

relative loss through reproductive support structures (capsule and stalk, about 20% vs. c. 10% in the other two species). *P. alpina*, however, lost about three times as much N (12%) as the other two species through its shed petals, which were both heavier (1.1 mg based on pooled samples, n = 146 petals) and contained more N (0.94 mmol g⁻¹) than those of *P. vulgaris* and *P. villosa* (0.9 mg, n = 95 and 0.2 mg, n = 47; 0.60 and 0.81 mmol N g⁻¹, respectively).

In 1996 there was little variation between species in total relative losses due to reproduction, ranging from 68% in *P. villosa* through 65% in *P. alpina* to 61% in *P. vulgaris* (Table 1). During 1997, flowering *P. villosa* lost considerably more N through reproduction (63%) than both *P. alpina* (45%) and *P. vulgaris* (51%).

N PRODUCTIVITY, MEAN RESIDENCE TIME AND N USE-EFFICIENCY

As with N pool size, dry matter productivity varied considerably between years in *P. alpina* (Table 2) but was relatively similar in 1996 and 1997 in the other two species. Despite the production of capsule and stalk, dry matter productivity was not significantly higher in reproductive than in non-reproductive plants (Table 3).

The annual average N pool size that tended to be smaller in flowering plants than in vegetative ones (Table 2), was similar in *P. alpina* and *P. vulgaris* (8– 10 μ mol N per plant) but was 60% smaller in *P. villosa*.

As a consequence of these patterns reproductive plants mostly showed a shorter mean residence time MRT of N and a higher aNP (Table 2). The lower N losses in *P. alpina* in 1996 than in 1997 reflected a relatively small decrease of N pool size between maximum and the winterbud stage, especially in non-reproductive plants. Non-reproductive plants showed an average MRT of 2.2 years (n = 6, SE = 0.4) across all species and years. Even if the extreme value for *P. alpina* in 1996 was excluded (average MRT 1.8 years, n = 5, SE = 0.2), mean residence time was still significantly higher than in reproductive plants (Table 3), which kept a unit of N for an average of only 0.8 years (n = 6, SE = 0.1).

N productivity tended to be higher in reproductive (mean: 1808 g mol N⁻¹ year⁻¹, SE = 279, n = 6) than in non-reproductive individuals (mean: 1042 g mol N⁻¹ year⁻¹, SE = 162, n = 6). However, this difference was not significant (P = 0.066, Table 3). Due to the different directions of variation of MRT and aNP, NUE did not differ between non-reproductive and reproductive plants (Table 3).

N RESORPTION EFFICIENCY AND RESORPTION PROFICIENCY

The leaf N pool of green leaves was 57% and 30% higher in reproductive *P. villosa* and *P. vulgaris*, respectively, than in non-reproductive plants (Table 4) but

Table 2 Annual dry matter productivity (mg year⁻¹), average nitrogen (N) pool size (µmol N plant⁻¹), N loss (µmol N plant⁻¹ year⁻¹), mean residence time (MRT, year), annual N productivity (aNP, g mol N⁻¹ year⁻¹) and N use-efficiency (NUE, g mol N⁻¹) of reproductive (R) and non-reproductive (NR) individuals of three subarctic Pinguicula species during 2 years (1996, 1997)

Year	Status	Productivity	Average N pool	N loss	MRT	aNP	NUE
Pinguicula	a alpina						
1996	NR	9.3	9.1	2.2	4.1	1022	4190
	R	8.6	8.4	6.0	1.4	1024	1434
1997	NR	12.6	8.1	4.7	1.7	1556	2645
	R	23.7	8.7	10.2	0.9	2724	2452
Pinguicula	a villosa						
1996	NR	1.9	3.9	2.5	1.6	487	779
	R	6.0	3.2	4.4	0.7	1875	1313
1997	NR	2.4	3.5	1.4	2.5	686	1715
	R	5.9	2.9	5.9	0.5	1113	557
Pinguicule	a vulgaris						
1996	NR	12.4	9.6	7.2	1.3	1292	1680
	R	15.0	8.9	11.9	0.7	1685	1180
1997	NR	10.9	9.0	5.0	1.8	1211	2180
	R	19.4	8.0	13.1	0.6	2425	1445

Table 3 The effects of species and reproductive status (reproductive, non-reproductive) on variables related to plant productivity and nitrogen (N) turnover in three carnivorous perennial herbs (Pinguicula alpina, P. villosa and P. vulgaris). Data in the table are F-values. Dependent variables are mean residence time (MRT), annual N productivity (aNP), N use-efficiency (NUE), average N pool size, dry mass (DM) productivity, annual N losses (N losses), N resorption efficiency (R_{EFF}) and N resorption proficiency (R_{PROF}). P-values were calculated using a permutation ANOVA approach (see Methods section). Degrees of freedom: species, 2; reproductive status, 1; interaction, 2; error, 6. Study years are replicates, thus n = 12

	Source of variation				
Dependent variable	Species	Reproductive status	Interaction		
MRT	1.569 NS	9.444*	0.319 NS		
aNP	1.239 NS	4.848 NS	0.075 NS		
NUE	6.054*	4.453 NS	0.840 NS		
Average N pool	182.884***	3.829 NS	0.829 NS		
DM productivity	6.094*	3.247 NS	0.039 NS		
N losses	11.999**	24.153**	0.916 NS		
R _{FFF}	12.342***	3.156 NS	0.071 NS		
R _{PROF}	4.746*	0.269 NS	0.387 NS		

NS = not significant (P > 0.05); *P < 0.05; **P < 0.01; ***P < 0.001.

Table 4 Leaf nitrogen pool (µmol N leaf⁻¹), nitrogen resorption efficiency (R_{EFF},%) and resorption proficiency (R_{PROF}, mmol N g⁻¹) of non-reproductive (NR) and reproductive (R) individuals of three species of Pinguicula during 2 years (1996, 1997). R_{PROF} denotes the N concentration of senescent leaves

Species	Year	Status	Leaf N	R _{EFF}	R _{prof}
Pinguicula alpina	1996	NR	1.05	38.1	0.79
o	1996	R	0.91	50.5	0.76
	1997	NR	0.57	33.3	0.63
	1997	R	0.63	38.1	0.57
Pinguicula villosa	1996	NR	0.62	41.9	0.85
0	1996	R	0.96	53.1	1.03
	1997	NR	0.46	56.5	0.97
	1997	R	0.73	56.2	1.06
Pinguicula vulgaris	1996	NR	1.60	70.0	0.78
0 0	1996	R	1.82	68.7	0.94
	1997	NR	0.93	53.8	0.63
	1997	R	1.36	73.5	0.55

differences were smaller in P. alpina (c. 10%). Leaf N resorption efficiency (R_{EFF}) differed significantly among species (Table 3) and was on average 40% in P. alpina, 51% in P. villosa and 67% in P. vulgaris and reproductive plants were at least as good as nonreproductive plants $(R_{EFF}(R) > R_{EFF}(NR))$ in four of six comparisons, Table 4). However, the effect of reproductive status on R_{EFF} was not statistically significant (Table 3).

N resorption proficiency was rather similar in P. alpina and P. vulgaris (0.68 and 0.73 mmol N g^{-1} , Table 4), but 30% higher in P. villosa (P = 0.0268, Table 3). Differences in R_{PROF} between reproductive statuses were generally rather small and showed no consistent pattern in P. alpina and P. vulgaris, but senescent leaves of P. villosa reproductive plants contained a higher N concentration than did non-reproductive plants (Table 4).

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Discussion

In general MRT and NUE of subarctic *Pinguicula* is comparable to published data on N retention of noncarnivorous herbs (cf. Eckstein *et al.* 1999). Thus the carnivorous study species share a similar N use strategy with other herbs and graminoids.

The photosynthetic NUE, i.e. the photosynthetic rate in relation to leaf N content, of the three *Pinguicula* species (Méndez & Karlsson 1999) shows similar ranking among species as the whole-plant NUE of the present study; *P. alpina* having *c*. 55% higher photosynthetic NUE than *P. villosa*. However, these carnivorous plants showed lower photosynthetic NUE than non-carnivorous species. Photosynthetic NUE is one component of the annual nitrogen productivity (Garnier *et al.* 1995) and thus part of a wider wholeplant-based NUE approach (Garnier & Aronson 1998).

There is considerable variation in temperature and precipitation between years in the study region (Holmgren & Tjus 1996) that may have an impact on plant growth, nutrient uptake and prey abundance for carnivorous species. Differences in N pool and dry mass productivity among years in the present study are probably related to this climatic variation. Since plant growth in high-latitude ecosystems may be constrained by a number of environmental factors such as N availability, temperature or precipitation (Chapin & Shaver 1985), the relative importance of these may also vary among years (e.g. Weih & Karlsson 1997).

THE EFFECT OF REPRODUCTIVE STATUS ON NUTRIENT TURNOVER

Owing to the loss of seeds, petals and senescent reproductive structures, N losses in reproductive individuals were two to three times higher than losses in non-reproductive plants (Table 1). In contrast, N losses due to leaves and budscales were relatively similar in reproductive and non-reproductive plants. These data confirm that in these plants reproduction considerably influences the N budget of the plant. This is significant especially in nutrient-poor habitats, where lost N can not easily be replenished by uptake from the soil. Can reproductive individuals compensate for this high N investment into reproduction by accumulating a higher average N pool or by resorbing nutrients more efficiently from senescing tissues than non-reproductive plants? In our study the average annual N pool of reproductive plants was smaller than that of non-flowering individuals in all three species (Table 2). Also during the actual growing season there was not a large effect of reproductive status on plant N pool size (Fig. 1). This is in line with the results of earlier studies, where only small differences in the allocation of biomass and N to somatic structures (leaves plus roots) between flowering and non-reproductive individuals of the study species was found (Karlsson 1986, 1988).

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Average R_{EFF} of the study species of between 40% and 67% (Table 4) matched with values found for a large number of plant species of different life-forms (Aerts 1996). No statistically significant differences in R_{EFF} between reproductive and non-reproductive plants were found (Table 3). However, there was a trend towards reproductive individuals resorbing equal or larger amounts of N from senescing leaves than nonreproductive plants (Table 4). Owing to the larger N content in green leaves of reproductive plants, their senescent leaves mostly showed equal or higher N concentrations (\mathbf{R}_{PROF}) than those of non-reproductive individuals (Table 4). Resorption from reproductive structures could not be estimated as N loss due to shed seeds could not be separated from possible N resorption from capsule and stalk. However, we may expect that N from reproductive structures would be allocated to developing seeds as these probably represent a strong sink for nutrients.

These observations do not provide any support for a compensation of higher N losses in reproductive plants through an increased N pool size or improved N resorption. This leads in turn to a considerably lower MRT in reproductive individuals as compared with non-reproductive plants (Table 2). In reproductive plants annual N losses were larger than the average N pool size so that each unit of N remained within the plant for less than 1 year (Table 2). It appears that such an impaired nutrient budget may have severe consequences on future survival and growth.

Within reproductive plants of the morphologically similar study species, the ranking in terms of reproductive allocation was consistent with the ranking of species in terms of NUE and its components (Fig. 2). Higher investment into reproduction thus led to both a



Fig. 2 Mean residence time (MRT, upper panel), annual nitrogen productivity (aNP, middle panel) and nitrogen use-efficiency (NUE, lower panel) in relation to reproductive allocation of three species of *Pinguicula* during 2 years. Open symbols = 1996; filled symbols = 1997; squares = *P. alpina*; triangles = *P. villosa*; circles = *P. vulgaris*.

lower MRT and aNP and resulted in a less efficient N use. Since MRT and aNP decreased with increasing reproductive allocation, there was no trade-off between these two variables among the study species. Our results thus shed some light on earlier empirical data on the effect of reproduction on growth, survival and fecundity. There are significant somatic costs of reproduction (RSC) in *P. alpina*, *P. villosa* and *P. vulgaris*, but these are smaller than the reproductive allocation of the species (RE) (Thorén *et al.* 1996).

The fact that RSC < RE in all species indicates that there should be some sort of compensation mechanism at work (Thorén et al. 1996). N uptake from soil or prey to meet the larger N requirements of reproductive individuals may be facilitated by higher prey trapping success of reproductive plants (vs. non-reproductive) of P. vulgaris (Karlsson et al. 1994). For P. alpina, its perennial root system that allows for rapid growth during the early part of the growing season when nutrients presumably are in good supply may compensate for some of the extra investment into reproduction. Furthermore, reproductive plants of P. alpina showed a higher photosynthetic NUE than non-reproductive individuals (Méndez & Karlsson 1999). However, the presumed compensation is not achieved through a higher N pool or N resorption from senescing leaves in reproductive plants. Hence, evidence for demographic costs of reproduction were found in all three species (Svensson et al. 1993) with survival probability considerably reduced in reproductive individuals.

P. villosa presents some sort of an unsolved paradox. This tiny plant possesses a small annual root system consisting of an average of two unbranched roots of a total length of 20 mm. Still it occurs in the most infertile habitat (on Sphagnum fuscum hummocks) and displays the highest reproductive allocation of the three study species. P. villosa shows the lowest frequency of repeated flowering of the study species and has the shortest population half-life (Svensson et al. 1993). There may be at least two explanations for the apparent paradox. (i) By allocating more resources to reproduction and increasing seed output at the expense of future survival and growth, species may escape these unfavourable conditions and approach a 'functionally' monocarpic life-history. (ii) Explanations for the evolution of reproductive allocation can not only be found at the individual or population level. Theoretical models predict that in a metapopulation context with local extinctions an evolutionary stable strategy should favour a high reproductive allocation in unproductive habitats (Ronce & Olivieri 1997).

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