



Leaf habit influences nitrogen remobilization in *Vaccinium* species

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Abstract

The effect of N supply on plant growth and leaf demography of a deciduous and an evergreen Ericaceae was studied in relation to their internal cycling of N. Mature ramets of *Vaccinium myrtillus* (deciduous) and *Vaccinium vitis-idaea* (evergreen) were established in sand culture for 1 year with an adequate supply of a balanced nutrient solution. During one growing season, the plants were given two levels of N supply enriched with ¹⁵N and eight sequential destructive harvests were taken. Recovery of unlabelled N in the new shoot was used to determine the remobilization of N from storage. Initially, growth was unaffected by N supply. After May, High N enhanced growth for both species but the nature of their growth response differed. For both species, new shoot biomass and leaf number increased but root biomass production was affected for *V. myrtillus* only. Whole plant biomass production was similar for both species under High N, but was greater for *V. vitis-idaea* under Low N. The amount of N remobilized to support new shoot growth was similar for the two species and was independent of N current supply. N was remobilized predominantly from previous year leaves for *V. vitis-idaea* and from previous year stems and roots for *V. myrtillus*. The contribution of remobilization to new shoot N was similar for the two species, but depended on N supply. Remobilization was faster in *V. myrtillus*, but lasted longer in *V. vitis-idaea*. The results are discussed in relation to species growth in N-poor environments, focusing on the extent to which species-differences in the

dynamic of N remobilization and growth may explain their adaptation to constant and/or changeable N supply.

Key words: *Vaccinium myrtillus*, *Vaccinium vitis-idaea*, deciduous, evergreen, heathlands, internal cycling of nitrogen.

Introduction

For perennial plants adapted to poor-N environments, selection is not only for high competitive ability for N acquisition but also for traits that reduce N losses (Aerts, 1999; Aerts and Van der Peijl, 1993; Eckstein and Karlsson, 1997). Evergreen species have been reported to be prominent in such environments (Monk, 1966; Chapin, 1980). Consequently, several comparative studies have attempted to understand the adaptive significance of leaf habit, i.e. evergreen versus deciduous, in relation to plant N conservation (Aerts and Van der Peijl, 1993; Gray, 1983). However, these studies have often involved comparison between species of different growth forms. As the relationships between plant growth and N status depend on leaf habit and growth form (Reich *et al.*, 1997; Cornelissen *et al.*, 1998), it might be more appropriate to compare species within the same growth form to distinguish the role of leaf habit from that of other morphological and physiological features associated with differences in growth form.

Reduction of N losses can be achieved by low rates of biomass turnover and/or efficient internal cycling of N. The rates of biomass turnover are lower for evergreen

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than deciduous species at the leaf level. However, at the level of the whole plant, biomass turnover is likely to depend more upon growth form than upon leaf habit, as it might be controlled by morphological features inherent to growth form such as the proportion of woody tissues or characteristics of the root system (Eckstein *et al.*, 1999). The internal cycling of N is the sum of the processes that allow the uncoupling of N acquisition from N utilization. It involves retranslocation, storage and remobilization of N from sites of storage to sites of utilization (Millard, 1988, 1996). These processes determine the proportion of N lost from the plant, and the proportions used for plant growth, maintenance and reproduction that are derived from N sources both internal and external to the plant. When trying to understand the effect of leaf habit on the internal cycling of N, most studies have emphasized the retranslocation of N from senescing leaves, focusing on its efficiency (Killingbeck, 1996; Eckstein *et al.*, 1998) or quantifying the amount of N lost through litterfall as a means of assessing the contribution made by internal cycling of N (Miller, 1986). This approach does not account for remobilization of N from evergreen leaves prior to senescence, which can represent a major proportion of the N cycled within the plant (Nambiar and Fife, 1991; Millard and Proe, 1993).

Another approach to study the internal cycling of N is the use of ^{15}N to distinguish N uptake within a growing season from N taken up in previous years and subsequently remobilized to support current growth. From the results of studies on several species (Deng *et al.*, 1989; Marmann *et al.*, 1997; Millard and Proe, 1993; Proe *et al.*, 2000; Thornton and Millard, 1993), it is apparent that sites of N storage depend on growth form and leaf habit and there can be great variation in the contribution of remobilized N to spring growth (30–93%). However, comparison between evergreen and deciduous species is difficult because measurements were taken at different times of the growing season or on different organs.

This paper compares the internal cycling of N for two wild, woody perennial shrubs belonging to the same genus, sharing the same growth form and adapted to similar, N-poor environments: *Vaccinium myrtillus* L. (deciduous) and *Vaccinium vitis-idaea* L. (evergreen). Using ^{15}N labelling, species-specific sites of N storage and the extent to which new shoot growth relied upon remobilization as opposed to root uptake were assessed. The plants were grown under two different levels of nitrogen supply to investigate the effect of leaf habit and N availability on both growth and internal cycling of N, in order to address the following questions: (i) does leaf habit affect the pattern of growth under low or high N supply and (ii) are differences in the internal cycling of N between the two species related to their different growth patterns.

Materials and methods

Experimental design and plant material

Ramets of *Vaccinium myrtillus* L. and *Vaccinium vitis-idaea* L. were collected in April 1996 in a semi-natural woodland (NVC community W17; Rodwell, 1991) in north-east Scotland (57°18' N, 2°33' W) and divided into sections of rooted rhizome bearing a single mature above-ground shoot. Ninety ramets of each species were planted in 3 dm³ pots and maintained in sand culture in a frost-free glasshouse until spring 1997. The pots were watered to field capacity once a week with a complete nutrient solution containing: NH_4NO_3 , 1.77; KH_2PO_4 , 0.66; K_2SO_4 , 0.08; NaH_2PO_4 , 0.1; MgSO_4 , 0.16; ferric citrate, 0.07 (mol.m⁻³) and CuCl_2 , 0.24; ZnSO_4 , 3.69; MnSO_4 , 3.69; Na_2MoO_4 , 0.04; H_3BO_3 , 9.55 ($\mu\text{mol m}^{-3}$); pH adjusted to 4.5 with H_2SO_4 . The nutrient solution composition was based upon that recommended by Ingestad for *Vaccinium* spp. (Ingestad, 1973) modified to supply N as ammonium nitrate (Millard and Proe, 1991). Between fertilizer applications, the pots were kept moist with acidified, deionized water (pH 4.5). In February 1997, at the start of budbreak, each pot was leached with 0.5 dm³ of deionized water followed by 1 dm³ of N-free nutrient solution to remove any remaining soluble N. The plants were transferred to a controlled environmental growth chamber. Photoperiod and temperature were changed weekly to reproduce the day length, maximum and minimum positive temperatures averaged outdoors the previous week, with a constant relative humidity of 80% and photosynthetic photon flux density of 300 $\mu\text{mol m}^{-2} \text{s}^{-1}$.

On 3 March 1997, six plants of each species were harvested and prepared for analysis as described below. The remaining plants were organised in six replicate blocks. Within a block, the plants of each species were allocated to either the High N or the Low N treatment. Pots were watered as described above, after adjusting the N concentration of the nutrient solution to either 5.5 mol N m⁻³ (High N treatment) or 2.75 mol N m⁻³ (Low N treatment). Nitrogen was supplied as $^{15}\text{NH}_4^{15}\text{NO}_3$ (enriched to an abundance of 3.438 atom% ^{15}N), in order to distinguish between N taken up by the plant before March and N taken up from March onwards. Before the switch from unlabelled to labelled N supply, the total number of buds was counted. Above-ground stems and leaves were marked at their extremity with a dot of enamel paint subsequently to identify tissues grown before and during the period of N labelling (i.e. current season growth).

Plant harvesting and partitioning

After the initial harvest, plants were harvested on 27 March, 30 April, 21 May, 3 July, 17 September, 19 November 1997, and 8 January 1998. At each date, one plant from each species and N treatment in each block was removed and separated into the following morphological compartments: the new shoot, i.e. current year growth divided into new leaves, new above-ground stems and any reproductive biomass such as reproductive bud, flower or berry attached to the plant at the time of harvest; the stems produced in the previous years, divided into green and woody (non-green) stems growing above- and below-ground (rhizome); the old leaves, i.e. the leaves produced the previous years (*V. vitis-idaea* only); the roots (irrespective of growth year) and the white below-ground stems sprouting from the rhizome. New and old (for *V. vitis-idaea*) leaves were counted. Total projected leaf area was determined using a Delta-T Area Meter (Burwell, Cambridge, England). All samples were then weighed, frozen into liquid nitrogen, freeze-dried, dry-weighed and milled before analysis.

Analysis of samples and data calculation

Determination of total N and ^{15}N concentrations were performed with a Tracer-MAT continuous flow mass spectrometer (Hemel Hempstead, UK). In any sample analysed, the amount of labelled N (derived from N taken up after March 1997) was distinguished from the amount of unlabelled N (derived from N taken up by the plant before March 1997) and calculated using the following equations:

$$N_l = N_t \times [(a - a_0)/(a_1 - a_0)] \\ \times [(14 \times (100 - a_1) + 15a_1)/(14 \times (100 - a) + 15a)]$$

$$N_u = N_t - N_l$$

Where N_t is the amount of N present in the sample, N_l and N_u are the amounts of labelled N and unlabelled N, respectively, expressed in unit weights. The abundance (atom% ^{15}N) in the sample and in the nutrient solution is given by a and a_1 , respectively. The abundance of plant N content prior to the start of ^{15}N labelling is given by a_0 and set to the ^{15}N abundance of atmospheric N_2 0.3663 atom% (Mariotti, 1983).

Data standardization

At the start of the labelling period, plant dry weight and N concentration ranged between 0.5 and 2.2 g dry matter, 0.8 and 1.5% N, respectively. To overcome the high variability between plants in initial size and N status, the data were transformed. Dry matter, nitrogen content, number of leaves, and total leaf area were scaled to that of a standard plant having the mean initial N content averaged across all plants, in order to distinguish between data variability induced by differences in inherent characteristics of individual plants, from the variability induced by treatments. The initial N content was assessed for each plant using the amount of unlabelled N per plant recovered at the time of harvest, after correction for the estimated amount of N which was lost since the start of the labelling period.

The amount of N lost since the start of the labelling period was estimated using the relationship between the initial number of buds per plant and N_{up} , i.e. the amount of unlabelled N recovered in each plant (significant at $P < 0.001$ within species). The effect of time (date of harvest) and N supply on the relationship for each species was tested using multiple linear regression (Genstat 5 Committee, 1993). Any significant variation at any date was assumed to indicate losses of N, in which case the initial N content per plant was calculated using the initial number of buds and the linear regression between initial number of buds and N_{up} established for plants harvested at all other dates ($n = 54$, $R^2 = 0.79$, $P < 0.001$). Otherwise, the initial N content was taken to be equal to N_{up} .

Initial N content averaged across all harvest and N treatments was similar for the two species (t -test, $\alpha = 0.05$), averaging 13.01 ($n = 84$, $SE = 1.02$) and 12.50 ($n = 86$, $SE = 0.67$) mg N per plant, for *V. myrtillus* and *V. vitis-idaea*, respectively. Thus, the mean value of 12.75 mg N per plant was used as an estimate of initial N content for both species.

Statistical analysis

Before statistical analysis, data were log, square root or angular-transformed where required to ensure normality of distribution and homogeneity of variance. Three treatment effects were tested: time (i.e. date of harvest), species (*V. myrtillus* versus *V. vitis-idaea*) and N supply (High N versus Low N). Tests of significance, calculations of means and least significant difference between means were performed using the Residual

Maximum Likelihood (REML) method (Genstat 5 Committee, 1993). The time-course of each variable was described and presented graphically using means and standard errors of means calculated through the REML procedure, after back-transformation where appropriate. The assessment of treatment effects at any date of harvest were based upon a comparison of means (transformed where appropriate) using the least significant difference between means ($\alpha = 0.05$).

Results

Growth in relation to N supply

Both species started the growing season with similar shoot and root dry weights (Fig. 1). New shoot growth had started by April for both species (Fig. 1b) while root growth did not start until late May (Fig. 1c). There was no effect of N supply on the timing or amount of growth up to the end of May, for either species. This period corresponded with the first flush of leaf growth for both species (Fig. 2a) and with the first period of flowering for *V. myrtillus* (Fig. 2b). By May, each species had produced a similar amount of new leaf dry matter and area under High N and Low N. Leaf number, specific leaf area (SLA) and mean area per leaf were unaffected by N supply at this time (Table 1). However, leaf growth differed between the two species. SLA was significantly lower ($P < 0.05$) for new leaves of *V. vitis-idaea* than for those of *myrtillus* but *V. myrtillus* produced three times more leaves than *V. vitis-idaea* (Table 1).

N supply altered the growth pattern of the two species later in the growing season, when the second flush of leaf growth occurred. For both species, growth stopped sooner under Low N compared to High N, as indicated by the fact that whole plant net biomass peaked earlier for Low N than High N plants (Fig. 1a). This was because increases in new shoot biomass (Fig. 1b), new leaf number (Fig. 2a) and reproductive biomass (Fig. 2b) stopped earlier in the season under Low N than under High N. For *V. myrtillus*, leaf abscission occurred between September and November with Low N, but between November and January with High N (Fig. 2a). Below-ground growth continued until the end of the experiment, irrespective of N supply or species (Fig. 1c), except for High N plants of *V. myrtillus*.

The extent to which N supply altered growth pattern after May (during the second flush of leaf growth) depended on the species. For *V. myrtillus*, N supply affected root growth as well as shoot growth. Increase in root biomass started between May and July under Low N, as opposed to after July under High N (Fig. 1c). From July until September, both root and shoot growth were enhanced for High N plants, leading to a maximum plant dry weight 95% greater than that of Low N plants. Leaf production was increased under High N through the growth of 118% more leaves per plants and mean

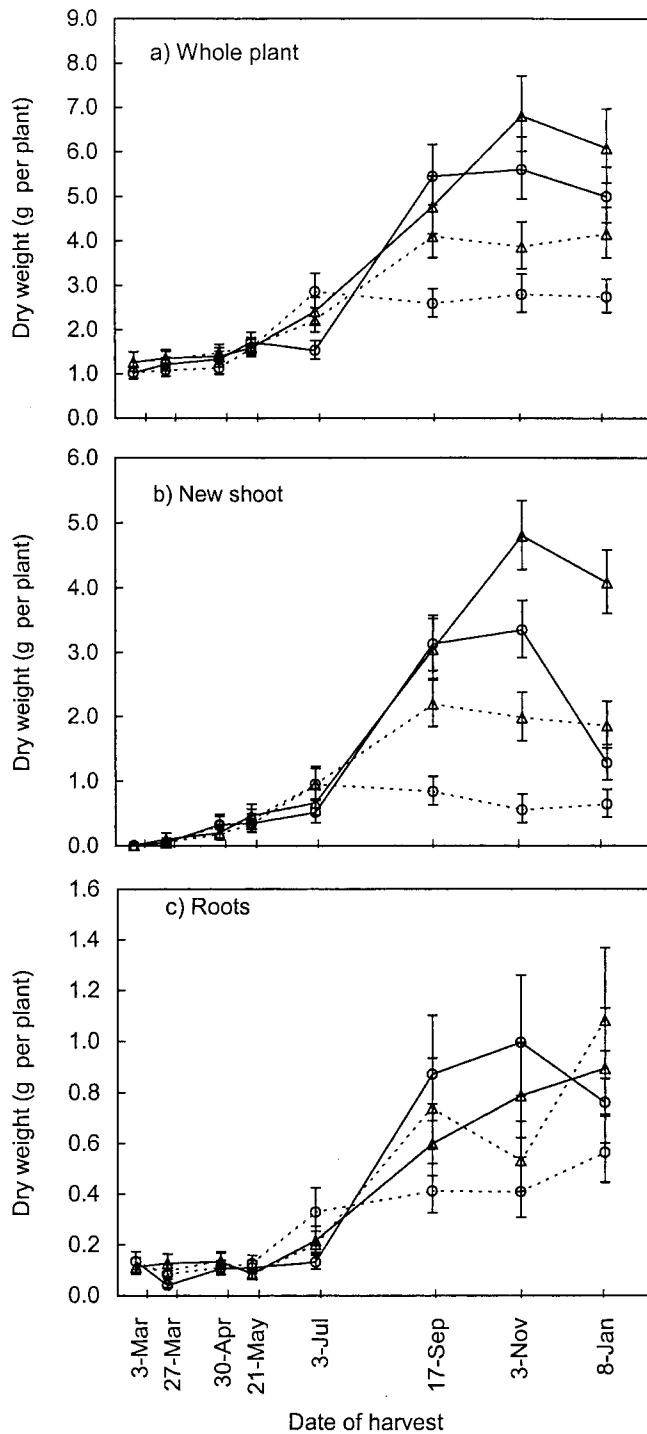


Fig. 1. The effect of N supply on whole plant, new shoot and root biomass. The symbols represent *V. myrtillus* (circles) and *V. vitis-idaea* (triangles) under High N (solid line) or Low N (dotted line). Values are the back-transformed means of 4–6 replicates from REML analyses. The vertical bars represent back-transformed SEM.

area per leaf larger by 75% (Table 1), with, however, no significant change of SLA.

In contrast, the seasonal maximum plant dry weight for *V. vitis-idaea* was 66% greater under High N than

under Low N, but N supply affected only shoot growth ($P < 0.05$) and had no effect on root biomass (Fig. 1b, c). Reproduction was enhanced under High N, with 13 times more reproductive dry matter produced by High N compared to Low N plants at the end of the experiment (Fig. 2b). The growth of new foliage was increased under High N, through the production of 168% more new leaves per plant. However, individual leaf traits (mean area per leaf and SLA) were not affected by N supply (Table 1).

The two species reached a similar ($P > 0.05$) seasonal maximum plant dry weight under High N (Fig. 1a). However, the seasonal maximum dry weight reached by Low N plants was 1.4 times greater for *V. vitis-idaea* than for *V. myrtillus* ($P < 0.01$), as biomass increment were sustained over a longer period in *V. vitis-idaea* than in *V. myrtillus*.

N remobilization and N uptake

The recovery of unlabelled N in the new shoot during the current growing season was used to measure remobilization of N to above-ground tissues. Remobilization was independent of N supply and both species remobilized a similar amount of N (Fig. 3a) averaging 5.4 ± 0.7 mg N and equivalent to 40% of plant N content at the start of the experiment. However, the period of remobilization was shorter for *V. myrtillus* than for *V. vitis-idaea*, since there was no significant increase of new shoot unlabelled N content after mid-May for *V. myrtillus*, but unlabelled N content of *V. vitis-idaea* new shoot continued to increase until mid-September. Therefore, the rate of remobilization, i.e. the rate of increment of unlabelled N in the new shoot, was higher for *V. myrtillus* than *V. vitis-idaea* (90 compared to 30 $\mu\text{g N per day}$, respectively).

Remobilization of N to the new shoot was paralleled by a significant decrease in unlabelled N content of roots and woody stems of *V. myrtillus*, but of old leaves and old non-woody stems for *V. vitis-idaea* (Table 2). Thus, N was remobilized from different morphological compartments in the two species. The main storage compartment appeared to be the old leaves (i.e. grown in previous years) in *V. vitis-idaea* as the decrease in their unlabelled N content accounted for 71% of the amount of unlabelled N recovered in the new shoot. The main storage compartments in *V. myrtillus* seemed to be the roots and woody stems, which accounted for 18% and 48% of the amount of unlabelled N recovered in the new shoot, respectively.

The amount of labelled N recovered at the level of the new shoot or the whole plant did not increase significantly from March to May for either species (Fig. 3b, c), indicating that rapid root uptake did not start before this time. Thus, for both species, the first flush of growth relied mainly on N remobilization, contributing $80 \pm 5\%$ and $55 \pm 5\%$ of new leaf N for *V. myrtillus* and *V. vitis-idaea*;

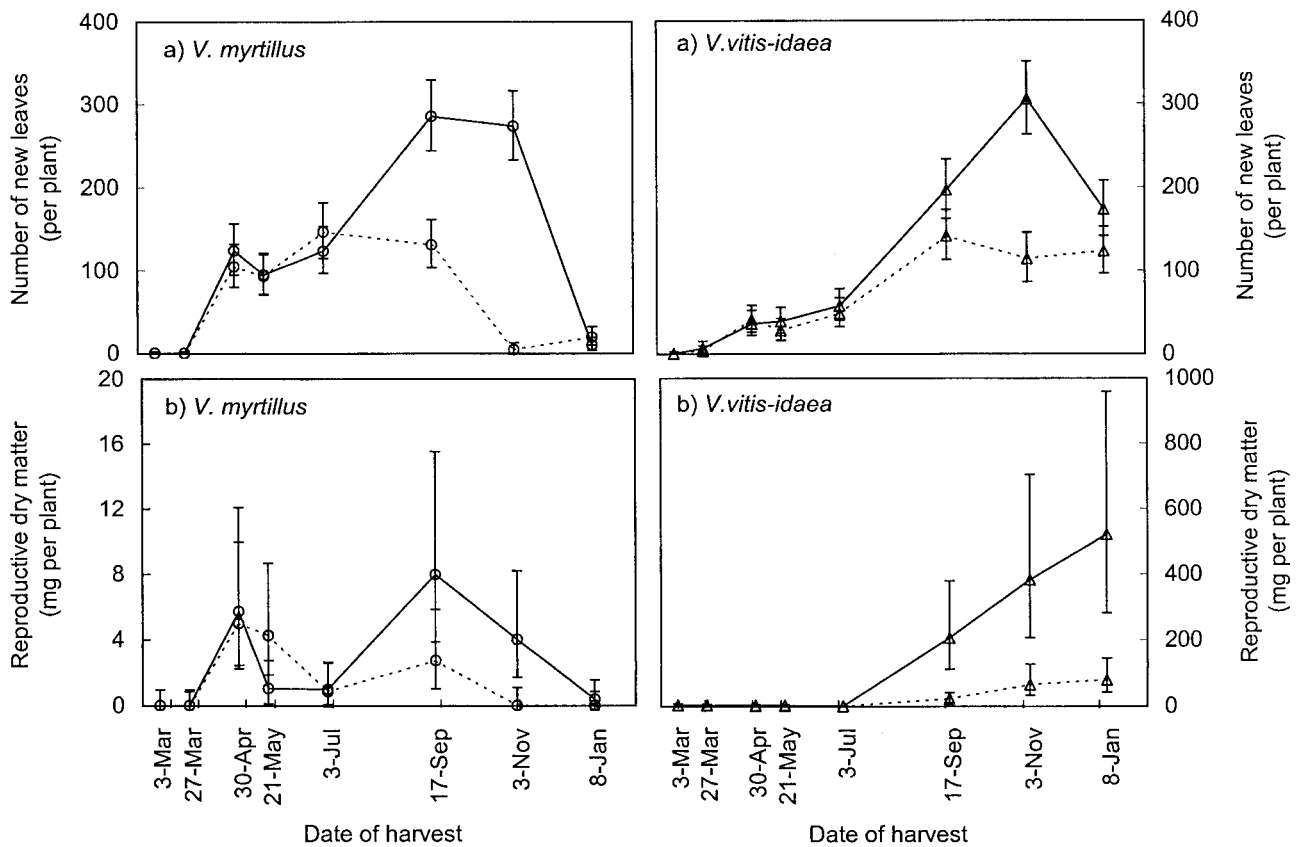


Fig. 2. The effect of N supply on new leaf growth (upper charts) and reproduction (lower charts). The symbols represent *V. myrtillus* (circles) and *V. vitis-idaea* (triangles) under High N (solid line) or Low N (dotted line). Values are the back-transformed means of 4–6 replicates from REML analyses. The vertical bars represent back-transformed SEM.

Table 1. The effect of N supply on new leaf number, total area, dry weight, specific leaf area and mean area per leaf at the end of the 1st and 2nd flush of growth

Values are the back-transformed means (\pm SEM) of 5–6 replicates from REML analyses. Means with different letters within a row are different at $P < 0.05$.

Parameters	End of first flush				End of second flush			
	<i>V. myrtillus</i>		<i>V. vitis-idaea</i>		<i>V. myrtillus</i>		<i>V. vitis-idaea</i>	
	High N	Low N	High N	Low N	High N	Low N	High N	Low N
Leaf number (per plant)	124 \pm 31 a	105 \pm 26 a	36 \pm 15 b	41 \pm 16 b	286 \pm 43 c	131 \pm 29 a	306 \pm 44 c	114 \pm 30 a
Total leaf area (cm ² per plant)	59 \pm 26 ab	54 \pm 21 ab	24 \pm 10 a	23 \pm 9 a	168 \pm 66 cd	45 \pm 18 ab	248 \pm 97 d	72 \pm 31 bc
Leaf dry weight (g per plant)	0.24 \pm 0.11 a	0.23 \pm 0.09 a	0.16 \pm 0.08 a	0.15 \pm 0.08 a	1.50 \pm 0.24 b	0.37 \pm 0.12 a	3.72 \pm 0.38 c	1.64 \pm 0.28 b
Specific leaf area (cm ² g ⁻¹)	247 \pm 25 a	237 \pm 21 a	158 \pm 14 b	156 \pm 14 b	125 \pm 11 b	129 \pm 12 b	68 \pm 8 c	46 \pm 7 c
Mean area per leaf (mm ² per leaf)	49 \pm 8 ab	52 \pm 8 abc	68 \pm 10 bc	59 \pm 9 abc	63 \pm 9 bc	36 \pm 5 a	83 \pm 13 c	65 \pm 11 bc

respectively. By 30 April, *V. myrtillus* had remobilized more N (5.0 ± 0.4 and 2.2 ± 0.5 mg unlabelled N per plant, for *V. myrtillus* and *V. vitis-idaea*, respectively; Fig. 3a) but had allocated less of it to each individual new leaf (32 ± 5 and 49 ± 8 μ g N per leaf for *V. myrtillus* and *V. vitis-idaea*, respectively), as more new leaves per plant were produced.

After May, the recovery of labelled N (derived from uptake) increased for both species and was dependent on N supply. Under High N uptake was sustained until

November by which time similar amounts of labelled N were recovered in plants of both species (Fig. 3c). Under Low N, N uptake did not continue after September, for either species. At that time 2-fold more labelled N had been taken up by *V. vitis-idaea* compared to *V. myrtillus* (Fig. 3c), leading to three times more labelled N recovered in the new shoot of *V. vitis-idaea* (Fig. 3b) because this species allocated more N to its new shoot than *V. myrtillus* ($44 \pm 5\%$ and $63 \pm 6\%$ for *V. myrtillus* and *V. vitis-idaea*, respectively). Consequently, by the end of the second

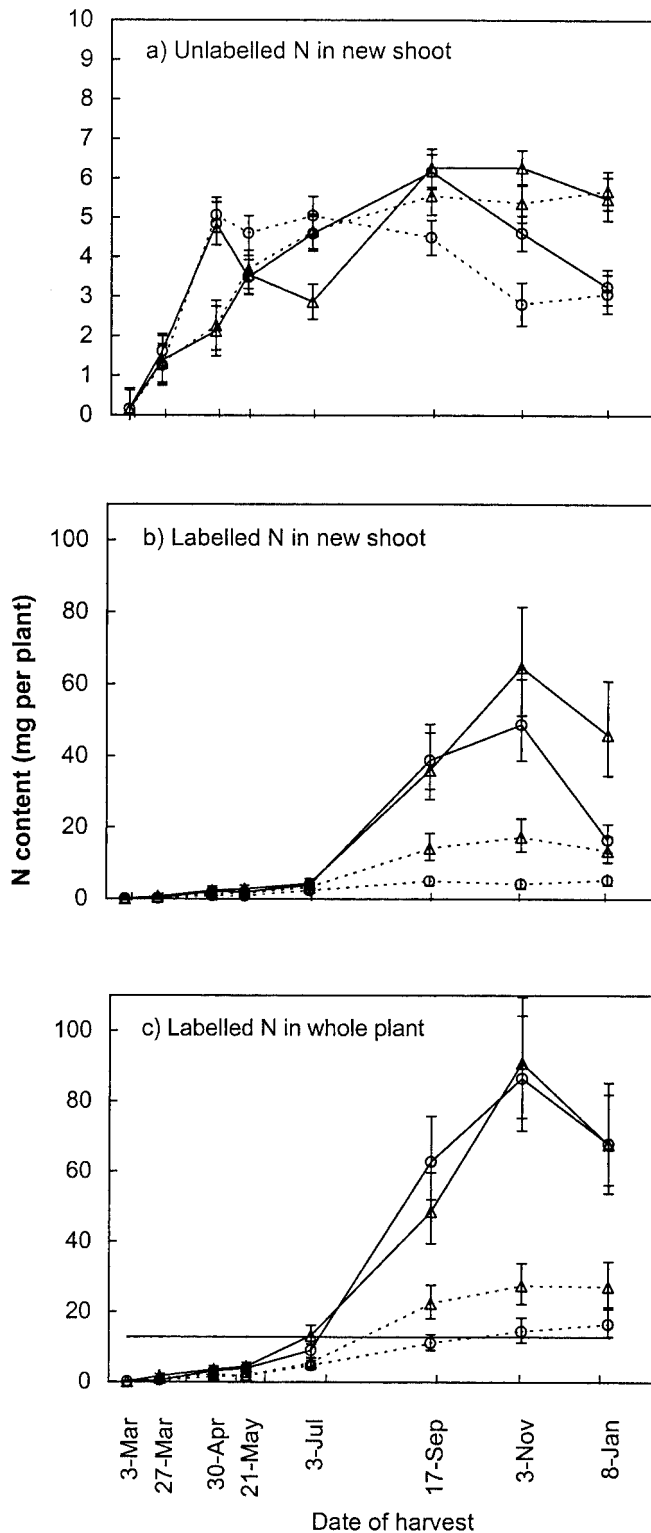


Fig. 3. The effect of N supply on the recovery of N derived from remobilization (unlabelled) in the new shoot and from uptake (labelled) in the plant. The symbols represent *V. myrtillus* (circles) and *V. vitis-idaea* (triangles) under High N (solid line) or Low N (dotted line). The horizontal solid line represents the initial N content (unlabelled N) per plant. Values are the back-transformed means of 4-6 replicates from REML analyses. The vertical bars represent back-transformed SEM.

flush of leaf growth, under High N less than 20% of the N in the new leaves was derived from remobilization for either species, while under Low N remobilization accounted for half of N in the new leaves of *V. myrtillus* but for only a quarter for *V. vitis-idaea* (Table 3). However, by the end of the growing season, the contribution of labelled versus unlabelled N to plant total N content was similar for both species and depended only on N supply (Table 3), with 82% and 62% of plant N derived from root uptake under High and Low N, respectively. The majority of this N had been allocated to the new leaves in *V. vitis-idaea*, and to the roots and stems in *V. myrtillus* (Table 4).

Discussion

Species growth response to N supply

Because the plants were grown in sand culture, mycorrhizal infection was insignificant and the plants probably relied solely on mineral N for uptake. However, the N status of our experimental plants, as assessed by their leaf N concentration, was similar to that reported by other authors for plants growing in the field (Table 5).

Under Low N *V. vitis-idaea* had a higher net biomass production than *V. myrtillus* (Fig. 1a). Therefore, over a single growing season, the evergreen species was better adapted to a constant low N availability. The greater biomass production achieved by the evergreen species did not rely on a greater contribution of N remobilization to growth, since the two species remobilized similar amounts of unlabelled N (Fig. 3a). However, for *V. vitis-idaea* remobilization was sustained over a longer period but at a lower rate than for *V. myrtillus*, and was coupled with a longer duration of growth and N uptake. Such a pattern of remobilization allows N to be supplied to the new shoot during the major part of the growing season, but restricts the ability to produce a large amount of biomass independently of external N supply. This highlights the necessity to account for duration as well as amount and rate of growth in order to understand how plants respond to environmental constraints.

At higher N supply, net biomass production of *V. myrtillus* doubled but only increased by 66% for *V. vitis-idaea*, compared to low N supply (Fig. 1a). This suggests that *V. myrtillus* is more responsive to changes in N supply and has an 'opportunistic pattern of growth', as defined previously (Gray and Schlessinger, 1983). Rapid but short remobilization of N can be consistent with opportunistic growth because it enables the plant to develop, in the short-term and independently of external N supply, the vegetative structures needed to take advantage of any subsequent increase in N external availability. This trait could be important in an environment where there are temporal fluctuations of N availability.

Table 2. The amount of unlabelled N (mg) recovered in the different compartments of *V. myrtillus* and *V. vitis-idaea* at the start (budburst) and at the end of N remobilization

Values are the back-transformed means \pm SEM of 4–6 replicates from REML analyses. Means for the end of remobilization correspond to unlabelled contents in plants harvested on 30 April and 17 September for *V. myrtillus* and *V. vitis-idaea*, respectively. Means with different letters for the same compartment are different at $P < 0.05$. The significance of the effect of time and N supply is given at $P < 0.001$ (***), $P < 0.05$ (*) and $P > 0.05$ (ns).

Species	Compartment	Budburst	End of remobilization		Significance	
			High N	Low N	N supply	Time
<i>V. myrtillus</i>	New shoot	0.2 \pm 0.5 a	4.8 \pm 0.5 b	5.1 \pm 0.4 bc	ns	***
	Old green stems	3.8 \pm 0.5 a	3.6 \pm 0.5 a	3.7 \pm 0.4 a	ns	ns
	Roots	1.2 \pm 0.5 a	0.3 \pm 0.3 b	0.5 \pm 0.3 b	ns	*
	Woody stems	4.6 \pm 0.8 a	2.6 \pm 0.6 b	2.1 \pm 0.5 b	ns	*
	Others	0.6 \pm 0.2 a	0.4 \pm 0.2 a	0.1 \pm 0.2 a	ns	ns
<i>V. vitis-idaea</i>	New shoot	0.1 \pm 0.5 a	6.3 \pm 0.5 c	5.6 \pm 0.5 bc	ns	***
	Old green stems	2.2 \pm 0.5 b	0.5 \pm 0.4 c	0.6 \pm 0.4 c	ns	*
	Old leaves	7.2 \pm 0.7 a	3.1 \pm 0.6 b	3.2 \pm 0.6 b	ns	***
	Roots	0.5 \pm 0.4 b	0.3 \pm 0.2 b	0.5 \pm 0.3 b	ns	ns
	Woody stems	1.6 \pm 0.5 b	1.4 \pm 0.4 b	1.6 \pm 0.4 b	ns	ns
	Others	0.0 \pm 0.2 a	0.2 \pm 0.1 a	0.0 \pm 0.1 a	ns	ns

Table 3. Unlabelled N as the proportion (%) of the total N content in the new leaves, new shoot and whole plant of *V. myrtillus* and *V. vitis-idaea*

Values are the means (\pm SEM) of 3–6 replicates from REML analyses. Means with different letters within a row are different at $P < 0.05$.

Time	Compartment	<i>V. myrtillus</i>		<i>V. vitis-idaea</i>	
		High N	Low N	High N	Low N
End of first flush	New leaves	72 \pm 4 a	87 \pm 4 b	47 \pm 4 c	64 \pm 4 a
	New shoot	71 \pm 6 a	87 \pm 5 b	47 \pm 7 a	65 \pm 7 a
	Whole plant	81 \pm 4 a	90 \pm 4 a	78 \pm 4 a	86 \pm 4 a
End of second flush	New leaves	18 \pm 4 ab	56 \pm 4 c	10 \pm 4 a	25 \pm 4 b
	New shoot	15 \pm 5 ab	48 \pm 5 c	9 \pm 5 a	23 \pm 5 b
	Whole plant	18 \pm 4 a	54 \pm 4 b	13 \pm 4 a	32 \pm 4 c
End of experiment	New shoot	23 \pm 6 ab	37 \pm 5 b	11 \pm 6 a	30 \pm 5 b
	Whole plant	20 \pm 4 a	41 \pm 5 b	16 \pm 5 a	32 \pm 5 b

Table 4. The proportion (%) of plant labelled N content allocated to the different plant compartments of *V. myrtillus* and *V. vitis-idaea* at the end of the experiment

Values are the back-transformed means (\pm SEM) of 4–6 replicates from REML analyses. Means within a row with different letters are different at $P < 0.05$.

Compartment	<i>V. myrtillus</i>		<i>V. vitis-idaea</i>	
	High N	Low N	High N	Low N
New leaves	3 \pm 5 a	2 \pm 10 a	48 \pm 5 b	38 \pm 5 b
New stems	17 \pm 2 a	20 \pm 3 a	7 \pm 3 b	4 \pm 3 b
Old green stems	14 \pm 3 a	15 \pm 3 a	4 \pm 2 b	3 \pm 2 b
Old leaves	–	–	5 \pm 1 a	2 \pm 1 b
Woody stems	17 \pm 4 ac	25 \pm 6 a	3 \pm 2 b	12 \pm 4 c
Roots	28 \pm 2 a	33 \pm 3 a	20 \pm 3 b	31 \pm 3 a
Others	17 \pm 5 a	0 \pm 0 b	11 \pm 4 ac	8 \pm 4 c

However, the extent to which growth will respond to increased N supply depends on the timing of N flushes in relation to the physiological stage of the plant, i.e. whether root uptake is possible.

Table 5. The concentration of N (mg g^{-1} DM) in the new leaves of *V. myrtillus* and *V. vitis-idaea* grown in this experiment and in their natural environment

<i>V. myrtillus</i>	<i>V. vitis-idaea</i>	Growth conditions	Reference
12–17	10–14	Sand culture	This study
17–18	10–13	English moorland	Cornelissen <i>et al.</i> (1996)
	8–15	Tussock tundra	Chapin and Shaver (1996)
10–12	17–22	Swedish heathland	Karlsson (1987)
14–20		Scottish heathland	Woodward (1986)

Long-term and short-term response to N supply

Several studies have already demonstrated that the amount of N remobilized depends on the amount of N in store (see Millard, 1996, and references therein). So if more N is allocated to storage, because N is available when the sink strength for growth is low, the amount of N remobilized during the subsequent growing season could be greater and lead to greater biomass production.

In their natural habitat, both species often experience flushes of soil N mineralization in spring and in autumn (Morecroft *et al.*, 1992; Williams, 1992). The latter part of the experiment, from September to January, was characterized by the cessation of vegetative growth above-ground for both species and leaf abscission for the deciduous one. This period, therefore, can be regarded as the equivalent of autumn, which would often correspond in the field with a flush of N mineralization. During this period, root uptake was possible for both species, as indicated by an increase of plant content in labelled N between September and January (Fig. 3). However, the allocation of this N to vegetative growth, reproduction or storage differed between the species.

For the deciduous species, since both reproductive and above-ground vegetative growth had stopped, N taken up in the autumn was allocated to the roots or the woody stems. This period corresponded with the seasonal maximal root biomass (Fig. 1) and at the end of the experiment, the major proportion of plant labelled N was recovered in the roots (Table 4). As this species stores N in its roots (Table 2), it is likely that an increase in N supply in autumn increases the amount of N stored and available for remobilization during the subsequent growing season, thereby having a long-term effect on growth rather than a short-term effect. Such a pattern of N allocation in autumn has been demonstrated for other deciduous species (Millard, 1996). In effect, autumnal N uptake may contribute up to 70% more to storage than spring uptake (Tagliavini *et al.*, 1999). However, when N supply is reduced in autumn the efficiency of N retranslocation prior to leaf senescence may increase (Millard and Thomson, 1989) and this would lead to a greater allocation to storage of the N taken up in spring and summer (i.e. during leaf production). Therefore, the manner in which *V. myrtillus* takes advantage of an increase of N availability in autumn would depend on the amount of N available earlier in the season. The level of N supply in spring and summer would affect the amount of leaf biomass produced (Table 1) and the amount of N available for subsequent retranslocation; while the level of N supply in autumn may affect both the amount of N retranslocated from senescing leaves and that taken up by the roots and allocated to storage.

For the evergreen species, a greater proportion of N taken up in the later part of the season was allocated to the new leaves under High N. This was indicated by an increase in area-based leaf N concentration between September and January (from 230 ± 18 to 289 ± 20 $\mu\text{g N cm}^{-2}$), no change in leaf area, and a concomitant increase in concentration of carbohydrates (G-A Grelet, unpublished data). Thus, an increase in N availability in autumn is likely to have increased the amount of N stored in the leaves and that subsequently available for remobilization in the following growing season(s).

However, the number of leaves produced and, therefore, the sink strength for N in autumn depended on the supply of N earlier in the season (between May and September, see Table 1).

The allocation of autumnal N uptake to reproduction also differed between the two species. Due to differences in amount of reproductive biomass produced, the proportion of N allocated to reproductive structures was four times higher for *V. vitis-idaea* than for *V. myrtillus* (averaged across both N treatments). For the evergreen species only it depended on N supply, with 2.6 times more N invested in reproduction under High N. This effect of N fertilization on reproduction has been reported for other evergreen species of the same growth form growing in similar environment (Wookey *et al.*, 1993). The functional significance of this trait is not clear, especially because these *Vaccinium* species rely mainly on vegetative reproduction and the rate of seed germination in the field is almost nil (Ritchie, 1955; Grime *et al.*, 1988).

These results suggest that neither species was able to take up N rapidly before the end of May (Fig. 3b). The extent to which they could take advantage of a flush of mineral N in spring would depend on its timing in relation to the start of root uptake (possibly the start of root growth) for the two species.

Sites of N storage

It was found that N was stored in *V. myrtillus* in the roots and the old woody stems (including the rhizome). Nordin and Näsholm showed that N concentration in the rhizome increased in autumn, due to a selective increase of free amino acids, providing indirect evidence that rhizomes may act as sites of N storage (Nordin and Näsholm, 1997). There is no evidence in the literature that N storage occurs in the roots of this species (mainly because the data available do not include the root system). However, for the deciduous *V. ashei*, it was shown that N storage in the roots accounted for 30% of the N remobilized for new shoot growth, while the rest was remobilized out of the stems (Birkhold and Darnell, 1993).

For *V. vitis-idaea*, it was found that most of the N was remobilized out of the old leaves. N storage in the leaves of evergreen trees have been reported for several species (Nambiar and Fife, 1991; Wendler *et al.*, 1995; Millard and Proe, 1993). Nordin and Näsholm showed that there was no autumnal increase of N concentration in the below-ground parts of *V. vitis-idaea*, and that an increase in plant N status did not lead to a change in N partitioning between the different N fractions quantified (Nordin and Näsholm, 1997). They suggested that N was not stored below-ground for this species and this is confirmed by the results of this study.

The ecophysiological significance of leaf habit for plant growth in N-poor environment

As far as is known, this paper presents the first study comparing, at the whole plant level, two species of the same growth form differing in their leaf habit. It shows that, over one growing season, both species utilize N from storage to the same extent, irrespectively of the level of N supply. This would imply that the ecophysiological significance of conserving N within the plant (i.e. storage) is the sequestration of N, which allows the amount of external N available for other species to be reduced. Such a trait can only be a selective advantage in N-poor environments if the species are highly competitive for N acquisition. The differences in the dynamic of N remobilization between the two species could explain the better adaptation of the evergreen species to grow under a constant low-N availability. Indeed its slower rate of remobilization enables growth to be sustained over a longer period within a year. However, through an opportunistic pattern of growth and a faster rate of remobilization, the deciduous species seemed more responsive to changes in N supply, which would be an advantage in its natural environment where N availability fluctuates seasonally. Therefore, to understand fully the effect of leaf habit on perennial growth, it is necessary to assess: (i) the long-term consequences of leaf-habit induced differences in the internal cycling of N and (ii) species response to the timing of changes in N availability as it occurs in their natural environment.

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