

Does morphology or the size of the internal N store determine how *Vaccinium* spp respond to spring N supply?

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Running title: Internal N and bud development in *Vaccinium* spp

Summary

1. *Vaccinium myrtillus* (deciduous) and *V. vitis-idaea* (evergreen) are ericaceous shrubs adapted to low nitrogen environments. Their comparative responsiveness to N supply was determined in relation to both N storage and developmental constraints.
2. Plants were grown with high or low N in sand culture to condition their N storage, and their growth measured during the first flush of a second year when plants from each treatment were again supplied either high or low N. ¹⁵N-labelling was used to quantify remobilisation of N taken up in the first year for growth in the second.
3. In both species, the growth response to external N availability was mediated through a change in the number of buds, initially present, which produced shoots, with no alteration of the number of leaves per shoot; but the magnitude of the response was smaller in the evergreen species. The second flush of growth took place more rapidly in *V. myrtillus* than in *V. vitis-idaea*, and depended on both external and internal N supply, in terms of the number of shoots produced and the number of leaves per shoot.
4. The amount of labelled-N remobilised by both species depended on the level of N reserves, and on the number of initial buds which produced shoots. In *V. myrtillus*, the total amount of N remobilised to new growth was significantly affected by external N supply. Since the total amount of N remobilised is independent of external N availability during spring growth, this result implies that the age of the N remobilised depended on the number of buds which produced shoots. We explain this result by the perennial nature of N storage in *V. myrtillus* and the age range of the pool of buds coming into growth each year on an individual plant.
5. N remobilisation and the growth response to N supply were closely linked with the pattern of bud activation. Species-specific growth responses to N supply in spring were better explained by developmental constraints on growth rather than by the ability to store and reuse N.

Keywords:

Bud phenology, Ericaceae, N remobilisation, N Reserves, ¹⁵N- Labelling.

Introduction

In temperate and cold climates, nitrogen (N) demand for new spring growth of perennial plants is met in part by root uptake (external N), and in part by the remobilisation of N from storage (internal N) (e.g. Aerts and Chapin, 2000; Chapin, Schulze and Mooney, 1990). The amount of N that is remobilised from storage is independent of the level of current N supply, and depends on the size of N storage pool, i.e. internal N availability, which itself depends on the level of N supply in the previous years (Millard, 1996). However, the extent to which N demand for new growth is met by storage or root uptake depends also on current N supply, i.e. external N availability (Grelet *et al.*, 2001; Millard and Proe, 1991).

The relative contribution of internal and external N to new growth also depends on the duration of N remobilisation. In some species, the remobilisation of N is fast and lasts for only a short period (less than 2 months). In that case, the tissues built during that period rely almost entirely on internal N, because root uptake during that time is slow. For example, in the fruit tree *Malus domestica*, Neilsen *et al.* (1997) found that 91 % of new growth N was derived from storage until the development of flowers, 45 days after bud break. This period corresponded to the growth of the spur leaves, the primary source of carbon for the developing flowers and young fruits, whose light interception in spring influences seasonal fruit yield (Wunshes and Lakso, 2000). In the wild species *Vaccinium myrtillus*, two months after budbreak, 88 % of new growth N was derived from storage (Grelet *et al.*, 2001). By then, all leaves and flowers produced during the first flush of growth had appeared, and buds were set on the new shoots for a second flush of growth (Grelet *et al.*, 2001). In this species, 80 % of all the leaves produced that year were grown during the first flush of growth, which produced 32 % of the maximum seasonal new growth biomass aboveground.

In other species the period of remobilisation can last 7 to 10 weeks, and contributes only 40-50% of new growth N. *Juglans regia*, *Prunus avium*, *Pinus sylvestris*, and *Betula pendula* display such a pattern of remobilisation (Frak *et al.*, 2002; Grassi *et al.*, 2002; Millard *et al.*, 2001). All these species produced between 60 and 100% of their maximal seasonal leaf area by the time remobilisation had finished.

Another wild species of *Vaccinium*, *V. vitis-idaea*, exhibits the longest duration of remobilisation yet reported in the literature (Grelet *et al.*, 2001). In this species, N remobilisation lasted for 6 months, at the end of which 15 to 28% of new growth N was derived from internal N, and 64 to 100 % of its new leaves had been initiated, when the availability of external N was high or low, respectively. Like *V. myrtillus*, *V. vitis-idaea* can produce more than one flush of growth per year, resulting from the growth of buds set in the previous flush, or from axillary buds after release of apical dominance. By the time all leaves from the first flush of growth were fully expanded, i.e. two months after bud break, only 64% of their N had come from storage, as remobilisation was only half completed (Grelet *et al.*, 2001). However, in *V. vitis-idaea* the number of leaves and the new shoot biomass produced during the first flush represented only 29 and 8 % of their seasonal maximum, respectively (Grelet *et al.*, 2001).

The effect of internal N availability on the new aboveground growth of a given species may depend not only on the duration of N remobilisation, but also on its

pattern of growth, including patterns of bud development, reproduction and leaf growth. Consequently, we hypothesise that the growth response of a species to external N supply will depend on internal N availability and on the developmental constraints on aboveground new growth, and that the effects of internal N availability are likely to be mediated through changes in initial growth at the start of the season.

This paper aims to assess the extent to which early season growth responses of *Vaccinium myrtillus* (deciduous) and *V. vitis-idaea* (evergreen) to external N availability depend on (1) developmental constraints on growth and (2) the availability of internal N. These two perennial species are closely related with very similar ecological niches (Rodwell, 1999). However, they differ in their growth pattern, as they have different leaf habit, with a stronger apical dominance in the evergreen *V. vitis-idaea* than in the deciduous *V. myrtillus* (Ritchie, 1955,1956). Both species produce a first flush of vegetative and reproductive growth in spring. Secondary flushes of growth can occur in both species, through the development of buds set during the first flush in both cases, and through the activation of dormant axillary buds in the case of *V. vitis-idaea*.

Both species have similar N requirements (Ingstad, 1973 Mäkipää, 1999). Furthermore, under a given level of N supply, both species remobilise a similar amount of internal N to support new growth (Grelet *et al.*, 2001). Yet they have been shown by several authors to differ in their sensitivity to the level of external N supply (Grelet *et al.*, 2001, Parson *et al.*, 1994; Press *et al.*, 1998). Different use of internal N, or different architectural constraints, might be the cause of these differences.

Materials and methods

EXPERIMENTAL DESIGN AND PLANT MATERIAL

Plants of *Vaccinium myrtillus* and *V. vitis-idaea* were obtained from a commercial nursery (Poyntsfield, Scotland UK) where they had been propagated and maintained outdoors for 2 years. In June during the first growing season of the experiment (year 1), they were washed, divided into sections of rooted rhizome bearing a single mature aboveground shoot and planted into 1.5 dm³ pots in sand. The plants were sorted into seven size-groups, according to the total volume of the aboveground growth. They were arranged on seven greenhouse benches according to their size, in alternating rows of each species. A natural photoperiod was used and the greenhouse was ventilated to provide temperatures close to ambient and kept frost free ($\geq 2^{\circ}\text{C}$). Seven replicate plants, one per bench, were allocated to each nitrogen treatment. The plants were watered to field capacity once a week with a complete nutrient solution containing either 5.5 (H) or 0.55 (L) mol N m⁻³, at the abundance of 3.78 atom % ¹⁵N. Other nutrients were supplied as described in Grelet *et al.* (2001). Between fertiliser applications the pots were kept moist with acidified, deionised water (pH 4.5). In mid-February of the following year, before the start of new growth in the second growing season (year 2), residual N was leached from the pots by the addition of 5 dm³ deionised water and 1 dm³ N-free nutrient solution. The plants that had received L and H in year 1 were supplied in year 2 with either 0.55 (L) or 5.5 (H) mol N m⁻³ at

natural abundance, resulting in four different N treatments in year 2 (LL, LH, HL and HH).

PLANT HARVESTING AND SAMPLE ANALYSES

The initial harvest was taken in early February of year 2, at the start of budbreak, before the switch from labelled to unlabelled N. The final harvest was taken at the end of July of year 2, six months after budbreak, by which time remobilisation was assumed to have finished (Grelet *et al.*, 2001).

At each harvest, one replicate plant per size class was randomly chosen from each treatment for both species. Plants were gently removed from the sand and washed with deionised water. Each plant was separated into: new above-ground growth produced during the 1st or the 2nd flush of growth, old stems i.e. stems produced during previous years, old leaves i.e. the leaves produced during previous years (*V. vitis-idaea* only), roots (growth from different years could not be distinguished) and white underground stems sprouting from the rhizome.

New leaves from the 1st and 2nd flushes of growth, and old leaves (of *V. vitis-idaea*) were counted. The number of new shoots produced in year 2 during the 1st and the 2nd flush of growth, the number of new inflorescences (*V. vitis-idaea*), together with the number of unbroken buds remaining on old stems of each plant were determined. The initial number of buds (IB) present on each plant at the start of year 2 was estimated by adding the number of new shoots or inflorescences on old stems to the number of unbroken buds remaining on the old stems. All samples were then frozen with liquid nitrogen, freeze-dried, weighed and milled before analysis. Determinations of total N concentration and ¹⁵N abundance were performed with a continuous flow-isotope ratio mass spectrometer (TracerMat Finnigan MAT GmbH, Bremen, Germany). For each sample, the amount of labelled N (derived from uptake during year 1) was calculated as described in Grelet *et al.* (2001). The recovery of labelled N in new shoot tissue that had grown from a bud during year 2 was used as a measure of N remobilisation.

STATISTICAL ANALYSIS

Calculations of means, least significant difference between means and tests of significance for the effects of species (Sp), Harvest date (H) and levels of N supply in year 1 (Yr1) and year 2 (Yr2) were performed by analysis of variance using Genstat software (Genstat 5 release 4.1). Where necessary, data were transformed using Log₁₀, square root, second power or ranking to satisfy the requirement for homogeneity of variance and normal distribution of residuals. When a log-transformation had been used, back transformed means were calculated after adding 1.15 * mean-square of residuals to each log-mean.

To correct for inherent variation in plant size, the number of buds present on each plant at the start year 2 (IB) was used as a covariate analyses of variance of dry matter, N contents, leaf and shoot numbers. Preliminary analysis of IB showed significant differences between species (P=0.005) but differences between harvests across the two species were not significant (P=0.51). However significant differences between means per species per harvest were found (P<0.001) (Table 1), which did not appear to have any biological explanation and were attributed to inherent variations of

IB across harvest for one or other species (despite replication across different size-groups). Consequently, the residuals of the two factor ANOVA (H X Sp) performed on IB were used as a covariate instead of the actual numbers of buds per plant (IB). Since the differences in IB between the two species could be explained by differences in plant architecture, the slope of the regression between variable and covariate was allowed to differ between species when ANOVAs were performed across species. The use of residuals as covariate has been proved to be useful in other cases when initial heterogeneity was difficult to measure (e.g. Marriott, Bolton & Duff, 1997).

Results

PLANT BIOMASS AND N STATUS AT INITIAL HARVEST

By the end of year 1, the level of N supply had not affected the biomass of either species (data not shown). However, N concentrations at the start of year 2 were 25% higher in both species for those plants which had N supplied at the higher level in year 1 (Figure 1). Thus two sets of plants, with contrasting internal N availability, had been produced for each species.

BIOMASS AND PLANT N STATUS AT FINAL BIOMASS

Biomass

V. myrtillus plants supplied with High N during either or both years produced 70% or 140% more biomass, respectively, than those supplied with Low N during two consecutive years (figure 2C). The N supply affected biomass production both above and below ground. Both new shoot growth and root biomass were enhanced to a greater extent when N was supplied at the higher level during two consecutive years as opposed to during the first or the second year only (Figs 2A and 2B).

When *V. vitis-idaea*, plants received High N in either or both years, the production of biomass at the whole plant level tended to be at least 25% greater than when the plants received only Low N (Fig. 2F). Supplying N at the higher level affected above and below-ground growth in a different manner, depending on when high N was supplied. New shoot growth was unaffected by the level of N supply in the first year (Fig. 2D), whilst root biomass tended to be 30% greater in the plants receiving High N in the first year compared to Low N (Fig. 2E). Conversely, High N supplied in the second year increased new growth two-fold (Fig. 2D), whilst it had no effect on root biomass (Fig. 2E).

N status

In both species, the N concentration in biomass depended only on the level of N supply in the second year, whether measured at the level of the whole plant, in the new shoots or the roots (Fig. 2, embedded charts). Therefore, plant growth response in the 2nd year to the previous year's N supply was mediated through a change in the amount but not in the N status of the biomass produced. The highest concentrations of

N were found in the roots in *V. myrtillus* and in the new growth in *V. vitis-idaea*, both of which are sites of N storage for the following year (Grelet *et al.*, 2001).

ABOVEGROUND MORPHOLOGY AT FINAL HARVEST

In *V. myrtillus*, high external N availability at the time of budbreak increased the proportion of buds which developed into a shoot (Table 2). This was manifest in year 2 both during the 1st and the 2nd flushes of growth. This might also explain why fewer buds remained on the plants at the start of year 2 (IB) when N was supplied at the higher level in year 1, as a greater proportion of buds probably developed into new shoots in year 1.

A high availability of internal N induced a greater proportion of buds to break in year 2, but only during the 1st flush of growth. The number of flowers produced per new shoot depended on both internal and external N availability. The number of leaves on 1st-flush shoots was unaffected by N availability, but on 2nd-flush shoots was greater under high external N. However, because the 2nd flush of growth had only just started when the plants were harvested and so the shoots were not yet fully expanded, this result could also mean that 2nd-flush shoots in low N plants were less advanced than in high N plants. As a consequence of a poorer 2nd flush of growth when availability of external N was low, the total amount of growth produced per growing point already present at the start of year 2 was smaller, both in terms of biomass and number of leaves. In addition, the availability of internal and external N affected mean leaf weight, which further explains the effect of N supply on new shoot biomass.

In *V. vitis-idaea* (Table 3), the proportion of buds breaking during the first flush of growth was marginally affected by the availability of external N. However, the proportion of buds developing into an inflorescence or a vegetative shoot and the number of leaves per vegetative shoot were similar in all N treatments. The 2nd flush of growth was still too premature in this species for full analyses, but preliminary observations suggested that a high availability of external N enhanced the number of 2nd-flush shoots that were developing.

REMOBILISATION OF STORED N

The recovery of labelled N in new growth allowed the amount of N remobilised from storage pools accumulated in year 1 to be quantified. The amount of labelled N remobilised per plant was greater in *V. myrtillus* than in *V. vitis-idaea*, irrespective of N supply (Fig. 3A). However, plants of both species remobilised 2.5 to 4.5 times more labelled N when N had been supplied at the higher level (Fig. 3A) in year 1. High N availability in year 2 significantly increased the amount of labelled N remobilised per plant in *V. myrtillus*, but not in *V. vitis-idaea* (Fig. 3A). Nevertheless, the amount of labelled N remobilised per individual new shoot was similar in both species and depended only on the availability of N in year 1, with no effect of the level of N supply in year 2 (Fig. 3B).

In *V. vitis-idaea*, all the labelled N was recovered in shoots grown during the 1st flush of growth only, whereas up to 28% of it was transferred to the shoots formed during the 2nd flush of growth in *V. myrtillus* (Table 4). In this species, high external

N availability significantly ($P < 0.01$) increased the proportion of new growth labelled N that was recovered in the 2nd-flush shoots (Table 4).

Discussion

GROWTH RESPONSE TO N SUPPLY

New growth tended to be more responsive to N supply in *V. myrtillus* than in *V. vitis-idaea*, both below and above-ground. Such a difference was probably due to seasonal growth being initially slower in *V. vitis-idaea* than in *V. myrtillus*, resulting in responses to N supply becoming apparent earlier in the season in *V. myrtillus*. At the whole plant level, the data were much more variable (standard errors equivalent to up to 25% of mean values) in *V. vitis-idaea* than in *V. myrtillus*. Although inherent plant heterogeneity might have been equally large for both species, less than one third of total plant biomass was invested in organs capable of primary growth (i.e. new shoots and roots) in *V. vitis-idaea*, compared to at least two thirds in *V. myrtillus*. Consequently, no significant effect of the treatments was observed on the biomass production of *V. vitis-idaea* plants, whereas *V. myrtillus* plants supplied with high N during both years produced more than twice the biomass of those receiving low N.

THE EFFECT OF N AVAILABILITY ON BUD GROWTH

N availability affected the production of new shoots through two different mechanisms: first, by the variation of the number of buds developing into a shoot; secondly by the alteration of the amount of growth produced by each bud. We found that both species responded to high external N availability by an increase in the number of buds developing into shoots. However the magnitude of this response was greater in *V. myrtillus* than in *V. vitis-idaea*, mainly because apical dominance is weaker in the former (Flower-Ellis, 1971; Ritchie, 1955,1956; Tolvanen, 1994a, 1995), where both apical and axillary buds can be activated.

The second mechanism involved in the morphological response to N supply was the variation of the amount of growth produced per growing bud. In our study, we focused on the effect of N supply on the growth of individual vegetative shoots (as opposed to reproductive), because the reproductive morphology differed too much between the two species to allow any comparison to be made. A vegetative shoot can be described as a suite of phytomers, the architectural units defined by White (1979) as being composed of an internode, a node, a leaf and an axillary bud. Brown and Sommer (1992) proposed the terms preformed and neofomed, to distinguish between phytomers formed during previous growing seasons that overwintered in the bud prior to their development, from those formed and extended during the current season. The number of phytomers preformed is affected by general level of fertility (e.g. *Fraxinus pennsylvatica*, Remphrey and Davidson, 1994a), including N status (e.g. *Picea sitchensis*, Chandler and Dale, 1995; *Prunus persica*; Lobit et al., 2001). Site fertility, together with the position of the bud within the canopy and along the parent shoot, influences the occurrence and magnitude of neofomed growth in several deciduous and evergreen genera, including *Arctostaphylos*, *Aesculus*, *Fraxinus*, *Nothofagus*, *Picea*, *Pinus*, *Quercus* and *Salix* (Brown and Sommer, 1992; Chandler and Dale,

1990; Davidson and Remphrey, 1994; Puntieri *et al.*, 2000; Stecconi, Barthelemy, 2002; Remphrey and Powell, 1984; Remphrey, Steeves and Neal, 1983; Remphrey and Steeves, 1984; Souza *et al.*, 2000).

In *V. vitis-idaea*, all the shoots developing during the first flush of growth of year 2 were produced by terminal buds, which were set in year 1. Since the number of leaves produced per 1st flush shoot was similar irrespective of N treatment, our data suggest that in *V. vitis-idaea* 1st-flush shoots were entirely preformed, so that the number of phytomers was fixed, irrespective of N supply.

In *V. myrtillus*, the buds growing during the first flush were distributed in the whole volume occupied by the aboveground system. If each bud was produced at the same time as the stem bearing it, the population of growing buds were of different age, i.e. the new shoots were produced by buds set during the course of several years preceeding and including year 1. In our study, we did not record the number of leaves produced per bud of each different age. Because N supply at the time of bud set might have differed from one year to the next, we could not assess the occurrence and magnitude of neoformed growth. in this species. In *Betula pubescens*, which exhibits a bud phenology similar to that of *V. myrtillus*, Lehtila, Tuomi and Sulkinoja (1994) found that latent, dormant buds make an even more important contribution to the bud population growth rate than vegetative, long and short shoots. However, there is no study published to date investigating the ability of such species to produce preformed or neoformed growth, or the extent to which these would be affected by N supply.

N REMOBILISATION

Within the new shoot

In *V. vitis-idaea*, the labelled N remobilised to above-ground, new growth was all contained in the tissues built during the first flush of growth. By contrast, in *V. myrtillus*, up to a third of labelled N was recovered in the tissues produced during the 2nd flush, and that proportion increased with the magnitude of the 2nd flush of growth, which itself depended on the availability of external N. Because the total amount of labelled N recovered in the biomass produced by each growing bud remained constant, irrespective of external N availability, this result suggests that some N was retranslocated from the tissues produced during the 1st flush to those built during the 2nd flush. Remobilisation of N from one flush to the next has previously been reported for *Pinus radiata* (Fife and Nambiar, 1984). This pattern of N redistribution within new growth is a feature of species exhibiting an indeterminate pattern of growth, where the number of flushes each year depends on environmental factors. Since most of *V. vitis-idaea* plants had not yet produced a 2nd flush of growth by the time of the second harvest, we can not reject the possibility that such a pattern of N redistribution also occurred in that species.

At the whole plant level

The results presented in this paper demonstrate that the amount of N remobilised by *V. myrtillus* and *V. vitis-idaea* depends, as in many other species, on the internal availability of N, i.e. the size of N stores (Millard, 1996). It was unaffected by external N availability in *V. vitis-idaea*, confirming findings reported by Grelet *et al.*

(2001) for the same species, and for many other herbaceous (Ourry, Boucaud and Salette, 1990) and woody species (Millard, 1996). By contrast, the amount of N remobilised by *V. myrtillus* did depend on external N availability. This result apparently disagrees with features of N remobilisation commonly reported for a wide range of species (see references above), including *V. myrtillus* grown in similar experimental conditions (Grelet *et al.*, 2001). In our previous study of N remobilisation by *V. myrtillus*, ^{15}N -enriched fertiliser was used to label current root uptake, while the unlabeled N recovered in the new growth was derived from the remobilisation of N stored in all preceding years (Grelet *et al.*, 2001). In the present study, ^{15}N -enriched fertiliser was used to label N taken up in year 1 and subsequently remobilised to support new growth. Hence the remobilisation of N derived from uptake in years preceding year 1 was not quantified. Therefore, in the present study, the external availability of N might not affect the total amount of N remobilised as such, but only the proportion of it that was derived from N stored *in* year 1 (the previous year), as opposed to that stored *before* year 1.

LINKING N REMOBILISATION TO BUD DEVELOPMENT

The amount of N remobilised depended on the number of buds which grew into shoots, and on N internal availability. We have previously shown that *V. myrtillus* stores N in both roots and stems, including the stem-borne, unbroken buds (Grelet *et al.*, 2001). The quantitative link between number of buds and amount of N remobilised might indicate that some of that N was stored in the bud itself, maybe in the preformed phytomers. Growth and N “deposition” in the phytomer is likely to be driven more by cell division than cell expansion (Brown and Sommer, 1992; Thornton *et al.* 1999; Schaufele and Schnyder, 2001). The greater amount of labelled N per shoot observed in plants with high N availability would then be explained by a greater number of cells per bud, with either more cells per preformed phytomers or more phytomers preformed in each bud. Because buds of *V. myrtillus* can be of different age, our results implies that (1) cell division occurred in year 1 in all buds set on the plant, irrespective of their age, and / or (2) the greater number of buds developing under high external N availability was accompanied by a shift in the mean age of the population of buds which grew into shoots. The first implication also suggests that N storage is partitioned in pools that are spatially and possibly chemically distinct, one pool being located in the resting buds, the other(s) found mainly in the roots (see Grelet *et al.*, 2001). There would then be both proximal and distal pool(s) of N storage, which would be significant with respect to tolerance of herbivory (see Millard *et al.*, 2001; Tolvanen, 1994b).

The second implication highlights the importance of understanding bud demography at the whole plant level. Tolvanen (1994a) reported that buds from the belowground stem started growth first and that budbreak was acrotonal. This implies that the buds borne by the older stems (OB, set up before year 1) were activated before those borne by younger stems (YB, set up in year 1). Because the pool of dormant OB is smaller than that of YB, the extra number of buds activated under high external N might be from the YB rather than the OB pool. More remobilisation with High. N could then have been due to the activation of a greater number of buds set in year 1, i.e. the youngest buds, linked with the remobilisation of the proximal stem / bud stored N. This shift in the mean age of the bud population emphasises the

substantial role played by the newest buds in determining shoot demography, as reported for another clonal shrub, *Salix arctica*, by Tolvanen, Schroderus and Henry. (2001).

To summarise, the two species, *V. myrtillus* and *V. vitis-idaea*, differed in the extent to which their growth was affected by internal or external availability of N. These differences can be explained primarily by species-specific patterns of bud activation and development, rather than by different patterns of N remobilisation.

The fate of N stored annually has previously been reported (Millard and Proe, 1991; Neiderholzer et al., 2001; Weinbaum and Van Kessel., 1998), but only for species setting and bursting buds over cycles restricted to one year (as opposed to several years in *V. myrtillus*). The bushy architecture of *V. myrtillus*, and the fact that the current year's shoot can develop from buds of different age provided a new insight on the perennial dimension of N storage in woody perennials, and on the aboveground source-sink relationship between growing tissues and stores of N. As far as we are aware, this paper presents the first study showing that temporal links exist between bud set and development on the one hand, and N storage and remobilisation on the other. The significance of these temporal links in terms of species-specific ecology and response to environmental changes remains to be fully appreciated.

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Tables**TABLE 1:** Initial number of buds in *V. myrtillus* and *V. vitis-idaea*.

Values are the means of 28 replicates. Means with different letters across rows and columns are different at $P < 0.05$.

Date of harvest	<i>V. myrtillus</i>	<i>V. vitis-idaea</i>
February	119 ± 14^c	56 ± 5^a
July	84 ± 8^b	96 ± 11^{cb}

TABLE 2: Morphology of the aboveground new growth in *V. myrtillius* at the final harvest (year 2).

Legends for N treatments as in figure 1. Values are means \pm SE, for 7 replicates, adjusted for covariate ⁽¹⁾ and back-transformed when data were transformed to their 2nd power ⁽²⁾, square-root ⁽³⁾ or LOG₁₀ ⁽⁴⁾ transformed prior to data analysis. Percentages budbreak were calculated as the ratio between the number of shoots and the number of buds present at the start of year 2 multiplied by 100. Means with different letters are different at P<0.05 within each row. Significant effects of the level of N supply in the first (Yr1) or second (Yr2) year of the experiment are indicated at P>0.1 (ns), P<0.1 (~), P<0.01 (*), P<0.05 (**), P<0.001 (***) and P<0.001 (***).

	N Treatments				Level of significance		
	HH	HL	LH	LL	Yr1	Yr2	Yr1 x Yr2
<i>New growth (1st and 2nd flush not distinguished)</i>							
Number of leaves per shoot	8.8±0.9 ^b	7.6±0.9 ^b	8.8±0.9 ^b	5.0±0.9 ^a	ns	*	ns
Shoot biomass (mg per shoot)	41±6 ^c	25±3 ^b	31±4 ^b	14±2 ^a	**	***	ns
Leaf weight (mg per leaf)	2.5±0.2 ^c	1.7±0.2 ^{ab}	2.0±0.2 ^b	1.5±0.2 ^a	*	**	ns
<i>1st flush of growth</i>							
Number of new shoots per plant ⁽¹⁾	53±16 ^{ab}	43±3 ^{bc}	56±11 ^a	35±2 ^c	ns	***	ns
Percentage budbreak ⁽²⁾	74±4 ^a	59±5 ^b	65±5 ^{ab}	38±8 ^c	*	***	ns
Number of leaves per shoot ⁽³⁾	5.3±0.5 ^a	5.4±0.5 ^a	5.2±0.5 ^a	4.4±0.5 ^a	ns	ns	ns
Number of flowers per shoot ⁽⁴⁾	1.7±0.1 ^a	1.25±0.1 ^b	1.2±0.1 ^b	1.15±0.0 ^b	**	***	*
<i>2nd Flush of growth</i>							
Percentage budbreak	20±3 ^a	13±3 ^{ab}	19±3 ^a	4±3 ^b	ns	**	ns
Number of leaves per shoot	4.7±0.5 ^a	4.2±0.5 ^{ab}	4.7±0.5 ^a	2.8±0.5 ^b	ns	*	ns

TABLE 3: Morphology of the aboveground new growth in *V. vitis-idaea* at the final harvest (year 2).

Legends for N treatments as in figure 1. Values are means \pm SE, for 7 replicates, adjusted for covariate ⁽¹⁾ and back-transformed when data were transformed to their square-root ⁽²⁾ or LOG₁₀ ⁽³⁾ prior to data analysis. ⁽⁴⁾ indicates that Analysis of Variance was performed on rank-transformed data. Percentages of budbreak were calculated as the ratio between the number of shoots and the number of buds present at the start of year 2 multiplied by 100. Means with different letters are different at P<0.05 within each row. Significant effects of the level of N supply in the first (Yr1) or second (Yr2) year are indicated at P>0.1 (ns), P<0.1 (~), P<0.01 (*), P<0.05 (**), P<0.001 (***) – indicates that statistical analysis could not be performed due to too few replicates.

	Means per nitrogen treatment				Significance of effects		
	HH	HL	LH	LL	Yr1	Yr2	Yr1 x Yr2
<i>New growth (1st and 2nd flush not distinguished)</i>							
Number of leaves per shoot	8.7 \pm 2.3 ^b	5.2 \pm 1.5 ^{ab}	5.5 \pm 1.5 ^{ab}	3.1 \pm 1 ^a	~	~	ns
Biomass of vegetative shoot (mg per shoot)	35 \pm 8 ^c	22 \pm 5 ^{ab}	30 \pm 7 ^{bc}	18 \pm 4 ^a	ns	*	ns
Leaf weight (mg per leaf)	2.7 \pm 0.4 ^a	2.5 \pm 0.4 ^a	2.8 \pm 0.4 ^a	3.4 \pm 0.5 ^a	ns	ns	ns
<i>1st flush of growth</i>							
Number of new shoots per plant ^(1,3)	18 \pm 4 ^a	12 \pm 3 ^{ba}	22 \pm 5 ^a	10 \pm 2 ^b	ns	*	ns
Percentage budbreak ⁽⁴⁾	21 \pm 7 ^a	12 \pm 2 ^a	18 \pm 3 ^a	16 \pm 3 ^a	ns	ns	ns
Number of leaves per vegetative shoot ⁽²⁾	7.3 \pm 0.7 ^a	7.1 \pm 0.6 ^a	6.7 \pm 0.8 ^a	7.2 \pm 1.2 ^a	ns	ns	ns
Proportion of shoots bearing inflorescences (%)	40 \pm 10 ^a	37 \pm 6 ^a	44 \pm 12 ^a	54 \pm 12 ^a	ns	ns	ns
<i>2nd Flush of growth</i>							
Number of plants exhibiting 2 nd flush	2	1	1	1	-	-	-
Number of shoots	13	11	14	6	-	-	-
Number of leaves per shoot	6.5	6.2	7.4	6.8	-	-	-

TABLE 4: Proportion of the amount of labelled N recovered in new growth, found in the 2nd-flush shoots .

Values are means \pm SE. for 7 replicates. N treatments as defined in text. Means with different letters are different at $P < 0.05$.

N treatments	Species	
	<i>V. Myrtillus</i>	<i>V. vitis-idaea</i>
LL	0 \pm 0 ^a	0 \pm 0 ^a
LH	28 \pm 11 ^c	0 \pm 0 ^a
HL	8 \pm 7 ^{ab}	0 \pm 0 ^a
HH	23 \pm 9 ^{bc}	0 \pm 0 ^a

1 **Figure Legends**

2 **Fig. 1:** whole plant N concentration at the start of year 2 (initial February harvest) of
3 *V. myrtillus* (●) and *V. vitis-idaea* (△). Plants were supplied with either a Low or High
4 level of N during year 1 and allocated to either the Low (LL or HL) or High (LH or HH)
5 level of N availability in year 2. Means with different letters are significantly different at
6 $P < 0.05$.

7 **Fig. 2:** New growth, roots and whole plant biomass and N concentration (inserts) at
8 final harvest (July, year 2). Within each chart, means with different letters are different at
9 $P < 0.05$. Legends for N treatments as in figure 1.

10 **Fig. 3:** Remobilised labelled N recovered in the new growth (A) or in the individual
11 new shoot (B) of *V. myrtillus* (□) and *V. vitis-idaea* (■). Vertical bars represent the
12 standard errors of means (A) or the least significant difference between means (B) at
13 $P < 0.05$. Means with different letters are different at $P < 0.05$. Legends for N treatments as
14 in figure 1.

Fig. 1

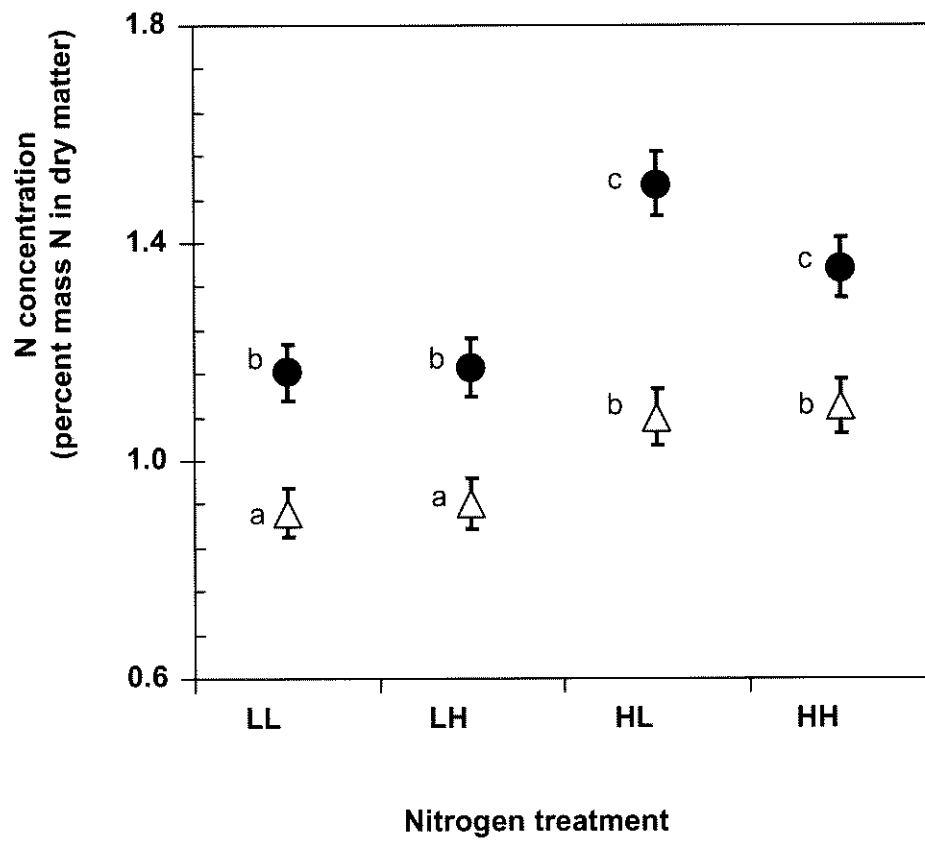


Fig. 2

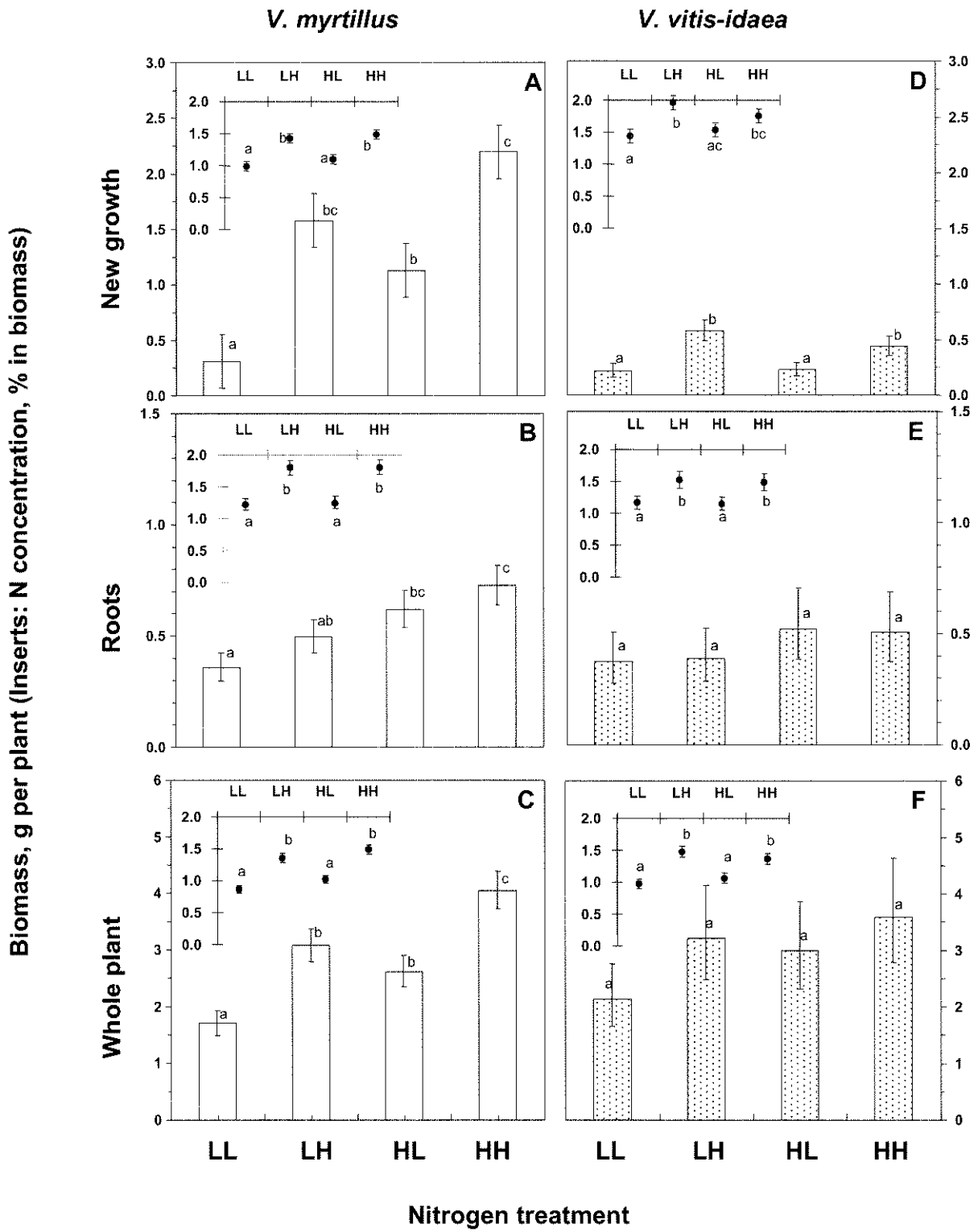


Fig. 3

