Relationships between phenology and the remobilization of nitrogen, phosphorus and potassium in branches of eight Mediterranean evergreens

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Summary

• Few studies have examined the effects of plant growth on nutrient remobilization in phenologically contrasting species. Here we evaluated the consequences of above-ground seasonality of growth and leaf shedding on the remobilization of nutrients from branches in eight evergreen Mediterranean phanaerophytes that differ widely in phenology.

• Vegetative growth, flower bud formation, flowering, fruiting, leaf shedding, and the variations in nitrogen (N), phosphorus (P) and potassium (K) pools in branches throughout the year were monitored in each species.

• Nitrogen and P remobilization occurred in summer, after vegetative growth and synchronously with leaf shedding. Despite the time-lag between growth and remobilization, the branches that invested more nutrients in vegetative growth also remobilized more nutrients from their old organs. Potassium remobilization peaked in the climatically harshest periods, and appears to be related to osmotic requirements.

• We conclude that N and P remobilization occurs mainly associated with leaf senescence, which might be triggered by factors such as the replenishment of nutrient reserves in woody organs, the hormonal relations between new and old leaves, or the constraints that summer drought poses on the amount of leaf area per branch in summer.

Key words: evergreens, leaf shedding, Mediterranean climate, nutrient remobilization, reproductive growth, vegetative growth.

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Introduction

The accumulation and remobilization of nutrients in the above-ground organs of perennial plants are highly dynamic processes (Marschner *et al.*, 1997). Nutrients are constantly imported into and exported from branches as a function of multiple factors (Hill, 1980; Lamaze *et al.*, 2003). Leaf age and the leaf shedding pattern (Del Arco *et al.*, 1991; Orgeas *et al.*, 2002), branch position within the crown (Niinemets, 1997), soil supply (Silla & Escudero, 2003) or growth requirements (Turner & Lambert, 1986) can affect the source or sink strength of a plant organ. In particular, nutrient remobilization, although controlled by many factors, ultimately contributes to optimizing the use of nutrients acquired (Marschner *et al.*, 1997).

The use of nutrients for growth constitute a major factor governing the dynamics of plant nutrient stores, and vice versa. Nambiar & Fife (1991) found that the amount of nitrogen (N) and phosphorus (P) remobilized (i.e. net decrease in the nutrient content of plant parts) was governed by the growth demands rather than by soil nutrient supply in *Pinus*. Accordingly, the nutrient requirements of *Picea* and *Pseudotsuga*

seedlings during spring vegetative growth are met by nutrient reserves, rather than by soil nutrient supply (van den Driessche, 1985; Proe & Millard, 1994). Even the nutrients of recently borne leaves of *Picea sitchensis* and *Pinus radiata* are withdrawn to supply second spurts of growth (Nambiar & Fife, 1987; Proe & Millard, 1994). By contrast, Silla and Escudero (2003) advocated that, in *Quercus* species, the contribution of plant nutrient stores to accomplish vegetative growth is important only when nutrient use exceeds soil supply. Reproductive events can also affect nutrient remobilization. The N content of *Rhododendron lapponicum* branches decreases during the period of reproductive activities (Karlsson, 1994). Similar relationships between reproduction and nutrient remobilization in fruit trees (Urban *et al.*, 2004), and in dioecious plants (Ågren, 1988) have been reported.

In addition to the nature of growth events (vegetative or reproductive), the seasonal distribution of these events also determines the pattern of internal nutrient use. Some studies have demonstrated the prevalence of nutrient remobilization to supply spring bud-break or vegetative shoot extension (Millard, 1994; Marmann *et al.*, 1997; Neilsen *et al.*, 1997; Kolb & Evans, 2002), and the relationships between nutrient resorption during leaf senescence and growth in evergreens (May & Killingbeck, 1992).

However, although the effects of vegetative growth upon internal nutrient dynamics have been extensively reported, comparatively little ecological research has addressed the investments in reproductive growth. Furthermore, most research on the relationships between phenology and nutrient remobilization in adult plants has been focused on short events such as the nutrient supply for spring bud-break or for vegetative shoot extension. Few studies have compared the chronology of both reproductive and vegetative growth, with that of nutrient remobilization along the whole growing season in more than two-three species in the field (cf. Mark & Chapin, 1989).

Mediterranean evergreens pose an additional challenge to understand the relations between phenology and the remobilization of nutrients from branch stores. Although nutrient remobilization can occur independently of leaf abscission in evergreens (Wendler *et al.*, 1995), a considerable amount of nutrients are remobilized during leaf senescence and shedding, which is frequently triggered by the growth of a new cohort of emerging leaves (Karlsson, 1994). However, in Mediterranean evergreens, spring–summer leaf senescence and shedding can be triggered either by growth demands or by summer drought (Munné-Bosch & Alegre, 2004). When leaf shedding is drought-induced, the remobilization of nutrients can be unrelated to growth.

Here, we compare the chronology of reproduction, vegetative growth, and leaf shedding, to the chronology of N, P, and K remobilization in the branches of eight Mediterranean evergreens growing in the field. We hypothesized that nutrient remobilization in the branches is regulated mainly by the occurrence of growth events, whether vegetative, reproductive, or both. On the basis of this initial hypothesis, we predicted the following patterns: (1) N, P and potassium (K) remobilization from old parts of the branch will be higher when reproductive and/or vegetative growth occurs, irrespective of the leaf shedding pattern, the suitability of the climatic conditions, or the nutrient under consideration; (2) the amount of N, P and K remobilized per gram of branch along the whole year should be directly related to the amount of N, P and K invested in current-year growth in that branch unit.

Materials and Methods

Study area and species

Eight evergreen phanaerophytes were selected for this study: *Arbutus unedo* L., *Bupleurum fruticosum* L., *Buxus sempervirens* L., *Cistus laurifolius* L., *Lonicera implexa* Aiton, *Pistacia lentiscus* L., *Quercus coccifera* L. and *Quercus ilex* ssp. *ballota* (Desf.) Samp. These species show a wide range of phenological patterns, as observed from previous work (Castro-Díez & Montserrat-Martí, 1998; Milla, 2005; Montserrat-Martí *et al.*, 2004). Each species was studied over two consecutive years from 1997 to 2000 (Table 1).

One population of each species was chosen in the western part of the Prepyrenean mountain range, north-east Spain (Table 1). The altitude of the sites ranged from 530 to 780 m above sea level. The sites are within an area of approximately 85 km^2. Before the growing season, at each site three horizon A soil samples were taken and analysed (mean pH 7.9; mean organic matter 4.2%; mean total N 0.1%; mean available P extractable with sodium bicarbonate (P-Olsen) 3.4 parts per million, and mean K-ammonium acetate 118.3 parts per million). Compared with other Mediterranean sites soil nutrient content was low (Specht, 1969; Rapp *et al.*, 1999).

The climate of the area is typically Mediterranean but it has a cold winter because of the continental character of the region. Average annual rainfall ranges from 630 to 824 mm. January is the coldest month (mean minima ranging from −1.2 to 3.6°C) and July–August are the hottest months (mean maxima ranging from 29 to 31°C) period.

To estimate the suitability of the climatic conditions when the nutrient remobilization events occur (hereafter, *Climate*), Turc's index of agroclimatic productivity was used (Turc & Lecerf, 1972). To estimate plant potential productivity in a given period, Turc's index takes into account precipitation, maximum and minimum temperatures, daylight seasonality, cloudiness, relative humidity, and climatic conditions in the preceding months. The index reaches a maximum when environmental conditions are most suitable for plant metabolism and growth, and a minimum when plant activity is strongly restricted by climatic conditions. Three reliable weather stations with long-term data were selected within the study area. Index values were obtained from agroclimatic records of the study area which contain the long-term series of the Turc's

Table 1 Average scores (mean ± 1 SEM, n = 10) of some phenological and nutrient remobilization parameters for the species of this study, together with their growth form, study years, and location of the study sites (UTM, Universal Transverse Mercator coordinates)

Species	Growth form	Study site (UTM)	Study year	*APS*	*PSI*	Nitrogen		Phosphorous		Potassium	
						$Nr_{BR(year)}$	$Ng_{BR(year)}$	$Pr_{BR(year)}$	$Pg_{BR(year)}$	$Kr_{BR(year)}$	$Kg_{BR(year)}$
Cistus laurifolius	Shrub	30T XM6397	1999	2.0 (0.0)	0.50 (0.00)	9.44 (0.32)	15.08 (1.03)	0.99 (0.04)	1.57 (0.11)	4.66 (0.13)	7.74 (0.52)
Cistus laurifollus			2000	2.0 (0.0)	0.50 (0.00)	9.11 (0.19)	11.20 (0.88)	1.02 (0.02)	1.33 (0.10)	4.94 (0.03)	7.52 (0.54)
Quercus ilex	Tree-(shrub)	30T XM8086	1997	6.5 (0.4)	0.52 (0.03)	4.17 (0.34)	8.09 (1.70)	0.77 (0.04)	0.61 (0.12)	4.85 (0.45)	4.74 (1.00)
Quercus ilex			1998	5.1 (0.9)	0.38 (0.02)	4.93 (0.28)	18.94 (2.28)	0.81 (0.03)	1.42 (0.20)	3.64 (0.18)	12.75 (2.40)
Quercus coccifera	Shrub	30T XM8285	1997	7.5 (0.2)	0.45 (0.03)	5.84 (0.75)	8.83 (2.06)	0.59 (0.07)	0.65 (0.17)	4.68 (0.58)	6.00 (1.71)
Quercus coccifera			1998	7.8 (0.2)	0.51 (0.03)	7.83 (0.54)	11.01 (1.34)	0.58 (0.03)	0.99 (0.11)	5.15 (0.19)	7.06 (0.80)
Arbutus unedo	Shrub-(tree)	30T XM8086	1997	9.7 (0.2)	0.78 (0.03)	4.44 (0.62)	6.96 (2.13)	0.47 (0.03)	0.67 (0.23)	3.71 (0.25)	7.10 (2.67)
Arbutus unedo			1998	10.2 (0.3)	0.87 (0.01)	9.38 (2.12)	13.79 (3.32)	0.83 (0.18)	1.27 (0.36)	8.89 (2.01)	13.09 (3.83)
Pistacia lentiscus	Shrub	30T XM8582	1997	6.1 (0.6)	0.93 (0.03)	7.40 (1.01)	11.29 (5.41)	0.59 (0.12)	0.81 (0.38)	4.06 (0.83)	8.20 (3.55)
Pistacia lentiscus			1998	6.6 (0.8)	0.92 (0.03)	3.10 (0.18)	5.96 (0.57)	0.66 (0.01)	0.51 (0.07)	5.70 (0.21)	5.32 (0.72)
Lonicera implexa	Vine	30T XM6682	1999	9.1 (0.4)	0.74 (0.01)	–	–	–	–	–	–
Lonicera implexa			2000	8.4 (0.4)	0.75 (0.03)	7.66 (0.62)	9.39 (0.87)	0.99 (0.34)	1.18 (0.34)	5.42 (0.53)	8.62 (1.00)
Bupleurum fruticosum	Shrub	30T XM6682	1999	9.1 (0.3)	0.97 (0.02)	–	–	–	–	–	–
Bupleurum fruticosum			2000	9.8 (0.2)	0.98 (0.01)	8.14 (0.86)	11.15 (1.57)	0.86 (0.27)	1.13 (0.28)	8.52 (0.92)	11.28 (1.63)
Buxus sempervirens	Shrub	30T XM8582	1997	7.4 (0.3)	0.7 (0.03)	5.79 (1.16)	8.30 (3.21)	0.38 (0.09)	1.00 (0.23)	4.93 (1.10)	4.85 (1.85)
Buxus sempervirens			1998	6.8 (0.5)	0.77 (0.01)	8.25 (1.14)	9.73 (4.44)	0.51 (0.08)	0.54 (0.23)	6.66 (1.31)	7.16 (3.37)

APS, active phenophasic period (months); *PSI*, phenophase sequence index (ratio); $Nur_{BR(year)}$, amount of nutrients remobilized in the whole year per gram of branch (mg g^{-1} branch); $Nug_{BR(year)}$, amount of nutrients in current year growth per gram of branch (mg g^{-1} branch). (See the Materials and Methods section for further descriptions of these variables.)

index for the three selected stations on a fortnightly basis (De León *et al.*, 1979). The long-term monthly averages (approx. 30 yr) of the Turc's index of the three stations were the monthly scores of the *Climate* variable. In our study area, the Turc's index peaked in late spring and dropped to its lowest values in mid-summer and in mid-winter.

Data collection

Phenology of growth, reproduction, and leaf shedding To study plant phenology, we used the qualitative method of Orshan (1989). Ten adult individuals per species were monitored monthly to assess visually the presence/absence of the above-ground resource-demanding phenophases (i.e. dolichoblast vegetative growth (*DVG*), flower bud formation (*FBF*), flowering (*F*), and fruit growth (*FG*)). *Cistus laurifolius* bears long (dolichoblasts) and short (brachyblasts) shoots, therefore one additional phenophase was assessed in this species: brachyblast vegetative growth (*BVG*). Each phenophase was recorded as present when more than 5% of the crown exhibited the phenophase in question.

To ensure that qualitative observations match real growth rhythms, the phenophases were also monitored quantitatively. One branch (tagged at the youngest stem without leaves: age of the tagged branch ranging from 2 yr old in *B. fruticosum* to 5 yr old in *B. sempervirens*) was randomly selected at the mid-crown of each of the 10 selected individuals per species. The organs attached to the branch (i.e. green leaves, senescent leaves, stems, flowers, inflorescences and fruits) from every cohort (current-year, 1 yr old, etc.) were counted monthly and recorded in a branch drawing. Dolichoblast growth dynamics, and formation and burst of reproductive buds in these branches were compared with the qualitative observations in the whole crown to determine the chronology of *DVG*, *BVG*, *FBF* and *F*. In addition, during the period of fruiting, a random sample of 50 fruits was collected from 20 to 40 plants per species monthly. Fruits were oven-dried and weighed, and the fruit mass-gain curves were used to check and determine precisely the *FG* period.

One litter-trap (19 cm internal diameter) was placed under the crown of each of the marked plants to monitor the phenology of leaf shedding (*LS*). Along the 2-yr study period per species, the leaf litter was collected monthly, oven-dried, and weighed.

To describe the phenological strategy of each species, we calculated two indexes. The length of the phenological cycle was measured using the Active Phenophasic Period (*APS*) index (Pérez-Latorre & Cabezudo, 2002). This index is defined as the number of months per year when any of the following phenophases are present: *DVG*, *BVG*, *FBF*, *F* or *FG*. The *APS* was calculated for each plant and study year. To estimate the degree of sequencing of vegetative and reproductive phenophases, the Phenophase Sequence Index (*PSI*; Castro-Díez & Montserrat-Martí, 1998), was used:

$$PSI = t(DVG + FBF + F)/[t(DVG) + t(FBF) + t(F)]$$

(t is the number of months required to complete the phenophase(s) in parentheses). This index ranges from 0.33 (phenophases overlapped) to 1 (phenophases performed sequentially).

Nutrient remobilization To determine the variations in branch nutrient concentration along the year, each month 15 mature individuals per species were randomly selected, avoiding the 10 plants selected to monitor phenology, and one sun-oriented, mid-crown branch per plant was collected. The branches were cut at the level of the youngest stem without leaves. Leaves, stems, flowers (or inflorescences), and fruits (or infrutescences) of the distinct cohorts were separated in the laboratory, and each fraction was pooled to obtain a single composite sample per organ cohort, and species. In the period of maximum leaf abscission, fully senesced leaves (senescent leaves detached easily with a gentle touch), were also harvested. The material was oven-dried to a constant weight at 60°C. The N concentration was measured with an elemental analyser (varioMAX N/CN; Elementar, Hanau, Germany), P concentration by vanado-molybdate colorimetry (Allen *et al.*, 1976) and K content with a flame photometer (Allen *et al.*, 1976). The marked branches used to monitor the demography of branch organs were also used to compute nutrient pools of the whole branch (see later). These branches are the experimental unit for our experimental design and the basis for the following calculations.

Test of prediction 1

Computing of variables The growth stage (hereafter, *Growth*) of the plants was classified as: NO-GROWTH when neither vegetative (*DVG* or *BVG*) nor reproductive (*FBF*, *F* or *FG*) growth occurred; or GROWTH: when either *DVG*, *BVG*, *FBF*, *F* or *FG* occurred. Similarly, the occurrence of leaf shedding (hereafter, *Leafshed*) was classified as: NO-LEAFSHED when the amount of litter collected in the litter-traps in a given month was less than 10% of the yearly maximum for a given species; or LEAFSHED when the litter collected in a given month was higher than 10% of that yearly maximum.

According to Marschner (1995), the term 'remobilization' is restricted in this article to describe the decrease in the net content of nutrients of plant parts during a given period, discarding losses caused by abscission of organs. The calculations described in this section take into account all year-round nutrient remobilization, measured on a monthly basis in the different leaf/stem cohorts separately. We assume that no remobilization occurs from senescing reproductive structures. To facilitate reading, the prefix Nu represents N, P and K, collectively; and N, P or K, represent a specific nutrient.

First, the net loss of nutrients from each leaf/stem cohort per month ($Nulost_{month}$) was computed as:

$$Nulost_{(month)} = (n\ M\ Nu_M)_{month-1} - (n\ M\ Nu_M)_{month}$$

(n is the number of leaves or stems per marked branch of leaf demography; M is the average dry weight of a leaf or an stem (taken from the 15 branches sampled for nutrient analysis), and Nu_M is the mass based concentration of N, P or K).

Second, the net loss of nutrients per branch unit over a month ($Nulost_{BR(month)}$) was calculated as:

$$Nulost_{BR(month)} = Nulost_{LEAVES(month)} + Nulost_{STEMS(month)}$$

where the $Nulost_{(month)}$ values for each leaf and stem cohort (excluding negative scores, i.e. those corresponding to nutrient accumulation) were added.

Third, the pool of nutrients shed from the branch through leaf abscission over a month ($Nushed_{BR(month)}$) was computed as:

$$Nushed_{BR(month)} = n_{sen(month)}\ M_{sen}\ Nu_{sen}$$

($n_{sen(month)}$ is the number of leaves shed per branch during a given month; M_{sen} is the average dry weight of a fully senesced leaf; and Nu_{sen} is the mass based concentration of N, P or K in the fully senesced leaves).

Fourth, the net remobilization of nutrients per branch unit over a month ($Nur_{BR(month)}$) was computed as:

$$Nur_{BR(month)} = Nulost_{BR(month)} - Nushed_{BR(month)}$$

Finally, in order to make the data comparable among species that differ in branch size, the monthly net remobilization values were standardized by transforming them to percentages of total yearly remobilization ($\%Nur_{BR(month)}$):

$$\%Nur_{BR(month)} = \frac{Nur_{BR(month)}}{\sum_{month=1}^{12} Nur_{BR(month)}}$$

To graphically compare the overlap between the nutrient remobilization events, the occurrence of phenophases, and the suitability of the climatic conditions (*Climate*), we calculated the percentage of species in a phenophase in a given month. Each species was weighted as a function of the number of individuals undergoing the phenophase. *Climate* and $\%Nur_{BR(month)}$ monthly values were also calculated, weighting $\%Nur_{BR(month)}$ values for each species as explained earlier. Next, all the variables were standardized so as to range from 0 to 1, and they were then plotted in a polar graph of radius 1 unit.

Statistics As phenology and leaf demography data were recorded on the same individuals, a set of 1176 (excluding outliers, and *L. implexa* and *B. fruticosum* in 1999, see later) paired data (7(10) individuals × 20(24) months × 6(8) species) was collected. To test whether *Growth*, *Leafshed* and *Climate* affected the net percentage remobilization of nutrients in a given month ($\%Nur_{BR(month)}$) three-way ANOVAs with *Growth*, *Leafshed* and *Climate* as fixed factors were run for $\%Nr_{BR(month)}$, $\%Pr_{BR(month)}$ and $\%Kr_{BR(month)}$ separately. *Growth* stages were classified as either GROWTH or NO-GROWTH. *Leafshed* was classified as either LEAFSHED or NO-LEAFSHED. Given that *Climate* is a continuous variable, we categorized *Climate* data in six discrete categories (1–6) in order to conduct the ANOVAs. These analyses allowed us to weight the relative contribution of the growth events, the phenology of leaf shedding, and the climate conditions to triggering nutrient remobilization.

Before the above procedures, the data set was checked for normality and homoscedasticity. Percentage data were transformed (arcsin[sqrt x]) to meet both requirements. To identify and discard multivariate outliers, multiple regression analyses including $\%Nr_{BR(month)}$, $\%Pr_{BR(month)}$ and $\%Kr_{BR(month)}$ as independent variables, and a randomized one as dependent variable, were performed separately for each species and phenological stage. The Mahalanobis distances for each case were calculated, as well as their probability to run out of a right-tail χ^2 curve. All the cases in which that probability was lower than 0.05 were considered as multivariate outliers (Hair *et al.*, 1998).

Test of prediction 2

Computing of variables The amount of nutrients remobilized per gram of branch along the whole year ($Nur_{BR(year)}$) was computed as:

$$Nur_{BR(month)} = \frac{\sum_{month=1}^{12} Nur_{BR(month)}}{M_{branch}}$$

(M_{branch} is the branch size (g) at the beginning of the growing season).

The amount of nutrients invested in current year growth per gram of branch along the year was calculated as:

$$Nug_{BR(year)} = \frac{n_{year} \times M \times Nu_m}{M_{branch}}$$

(n_{year} is the number of leaves, stems, flowers (or inflorescences), or fruits (or infrurescences) produced in a growing season per marked branch of leaf demography; M is the average dry weight of each element at full maturity (taken from the 15 branches sampled for nutrient analysis); and Nu_M is the mass based concentration of N, P or K). We considered separately (1) the total amount of nutrients in current-year growth ($Nug_{BR(year)}$), (2) the amount of nutrients in current-year vegetative structures ($Nugveg_{BR(year)}$) and (3) the amount of nutrients in current-year reproductive structures ($Nugrep_{BR(year)}$).

Statistics Simple linear regressions between $Nug_{BR(year)}$, $Nugveg_{BR(year)}$ and $Nugrep_{BR(year)}$ vs $Nur_{BR(year)}$ were run for N, P and K separately. Normality, homoscedasticity and outliers detection were handled as explained earlier.

Leaf demography data were missing for *L. implexa* and *B. fruticosum* in 1999. Therefore, the results refer to six species during the first study year, and to eight species during the second. All the statistical analyses were performed using SPSS 11.0 (SPSS, Inc., Chicago, IL, USA).

Results

Seasonality of growth, leaf shedding and nutrient remobilization

The phenological variety of the eight study species can be arranged in a gradient between the following extremes: species that tend to concentrate their phenology in a short period, allowing overlap among vegetative and reproductive phenophases (low *PSI* and *APS*) and species that tend to protract their activity throughout the year, avoiding overlap (high *PSI* and *APS*) (Fig. 1). Along this gradient, *C. laurifolius* was at the extreme of short cycle and high overlap of phenophases, while *B. fruticosum* and *L. implexa* were the species that protracted more their activity along the year.

The growth of vegetative shoots (*DVG*) peaked in mid-spring and all species stopped shoot extension by the beginning of the summer. The abscission of old leaves (*LS*) occurred throughout the summer. Reproductive investments were more scattered throughout the growing season, and only winter was clearly avoided. Flower development (*FBF* and *F*) tended to occur in spring, while fruit growth (*FG*) was attained during summer–autumn (Fig. 2). Details on the phenology of the individual species can be found elsewhere (Milla, 2005). The seasonal distribution of nutrient remobilization was very similar for N and P, occurring mainly in the summer. In contrast, several events of K remobilization were detected along the year (Fig. 2).

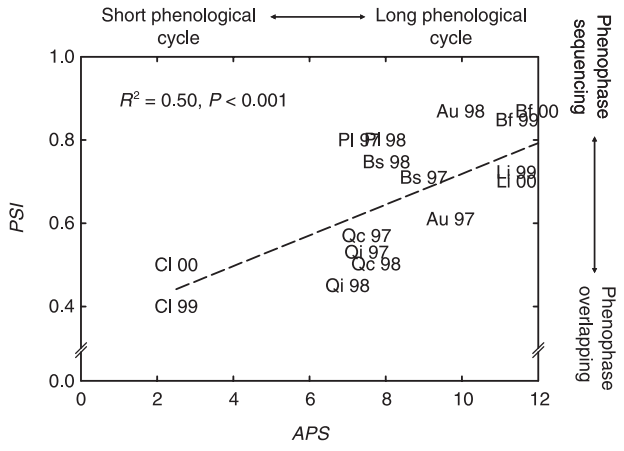

Fig. 1 Relation between the degree of phenophase sequencing (*PSI*), and the length of the phenological cycle (*APS*) in the eight species of this study. Species with high *PSI* are those that perform vegetative growth, flower formation, and anthesis one after another, and species with low *PSI* those that overlap such phenophases. Au, *Arbutus unedo*; Bf, *Bupleurum fruticosum*; Bs, *Buxus sempervirens*; Cl, *Cistus laurifolius*; Li, *Lonicera implexa*; Pl, *Pistacia lentiscus*; Pt, *Pistacia terebinthus*; Qc, *Quercus coccifera*; Qf, *Quercus faginea*; Qi, *Q. ilex* ssp. *ballota*.

Regarding the dependence of monthly nutrient remobilization on the phenological stage (prediction 1), we found little evidence for a direct, straightforward control of reproductive and vegetative activities upon nutrient remobilization. There was some overlap between $\%Nr_{BR(month)}$, and $\%Pr_{BR(month)}$, and growth events such as the end of *DVG*, the blossom of some late-flowering species such as *B. fruticosum* or *C. laurifolius*, or the growth of fruits of *B. sempervirens* or *P. lentiscus*, which mature in summer. The $\%Kr_{BR(month)}$ also overlapped with most fruit growth (Fig. 2). However, $\%Nr_{BR(month)}$, and $\%Pr_{BR(month)}$ were not significantly lower for NO-GROWTH than for GROWTH stages, and $\%Kr_{BR(month)}$ was only slightly higher in GROWTH stages (mean value for GROWTH = 0.11; mean value for NO-GROWTH = 0.08) (Table 2). Thus, *Growth* exerted a weak effect compared with *Leafshed* or *Climate* (Table 2). In addition, the variation in the nutrient remobilization percentages during each phenophase (*DVG*, *FBF*, *F* and *FG*) was extremely high (CV ranging from 73 to 188%, data not shown). Third-order interactions were not significant, therefore they were not included in the models.

By contrast, the occurrence of N and P remobilization was tightly coupled to the phenology of leaf shedding (Table 2, Fig. 2). On a seasonal basis, both processes were nearly simultaneous, extending from late-spring to late-summer, and peaking in mid-summer. This was reflected in the quantitative analyses: the F-Snedecor scores for the *Leafshed* factor were 2–30-fold higher than those of *Climate* or *Growth* in the ANOVA models with $\%Nr_{BR(month)}$ or $\%Pr_{BR(month)}$ as the dependent variables (Table 2). However, K remobilization was not so clearly governed by the leaf shedding pattern, reflecting some independence between K remobilization and senescence-induced nutrient remobilization.

Climate was more important for regulating the intensity of remobilization than *Growth* (Table 2). Although the effect of *Climate* upon N and P remobilization was more modest than that of *Leafshed*, it is remarkable that, in the harshest periods of the year – mid-summer and mid-winter – plants exhibited the highest and the lowest $\%Nr_{BR(month)}$ and $\%Pr_{BR(month)}$, respectively (Fig. 2). The $\%Kr_{BR(month)}$ also showed a peak in mid-summer, although some additional secondary peaks were detected in mid- to late winter, and at the beginning of autumn. In addition, K remobilization was low during the spring (Fig. 2). Therefore, K is more intensely remobilized in climatically harsh periods of the year.

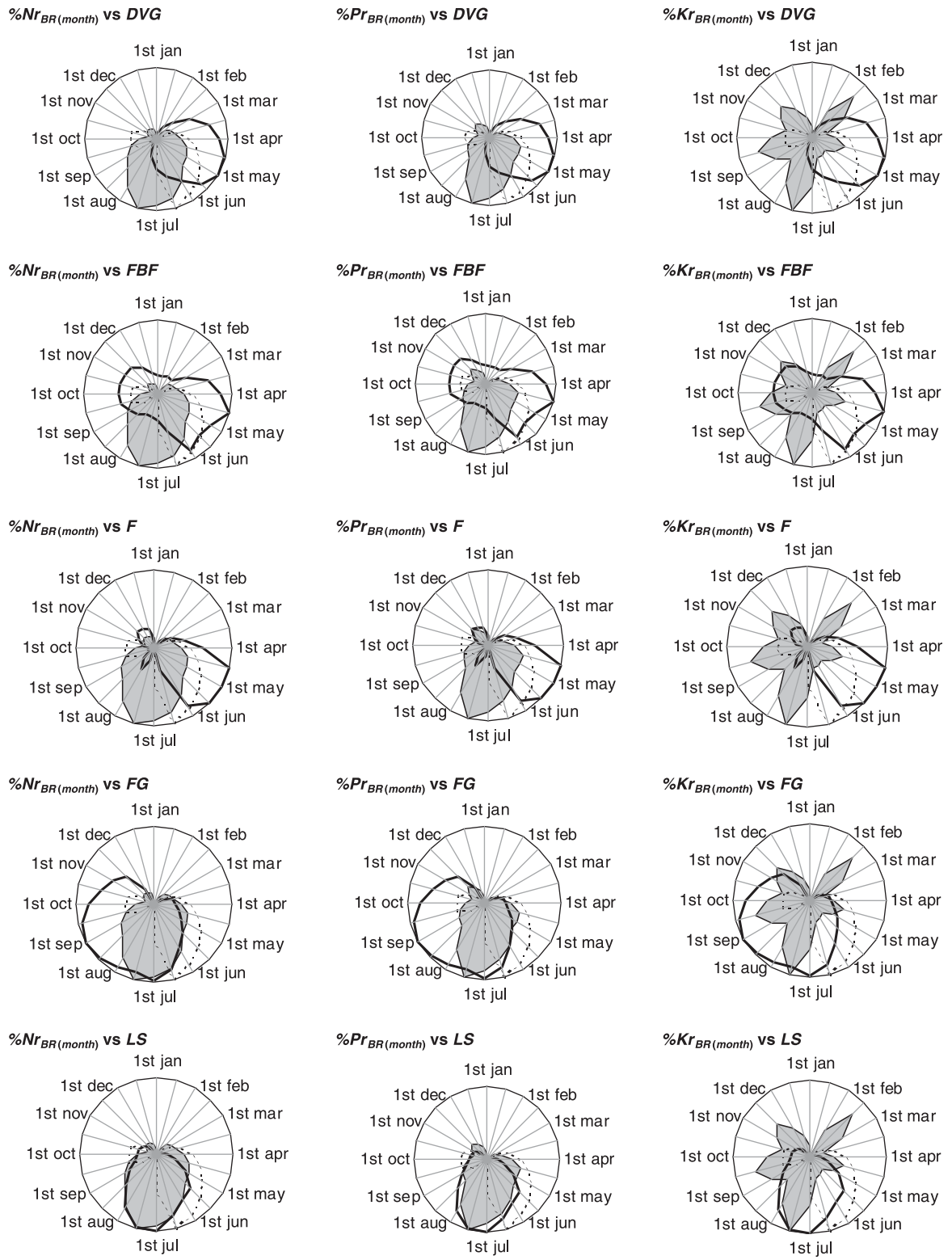

Fig. 2 Degree of overlap between the remobilization events (%$Nr_{BR(month)}$), the occurrence of phenophases (*DVG*, *FBF*, *F*, *FG* and *LS*), and the suitability of the climatic conditions (*Climate*) along the year. The phenophases are the empty areas with solid line, *Climate* is the empty area with dashed lines and the remobilization events are the filled (grey) areas. *DVG*, dolichoblast vegetative growth; *FBF*, flower bud formation; *F*, flowering; *FG*, fruit growth; *LS*, leaf shedding. %$Nr_{BR(month)}$, %$Pr_{BR(month)}$ and %$Kr_{BR(month)}$ are monthly net nutrient remobilization (see the Materials and Methods section for details).

	$\%Nr_{BR(month)}$		$\%Pr_{BR(month)}$		$\%Kr_{BR(month)}$	
	F	*P*	*F*	*P*	*F*	*P*
Growth	3.1	0.08	1.0	0.31	8.4	< 0.01
Leafshed	63.5	< 0.001	31.3	< 0.001	13.3	< 0.001
Climate	21.1	< 0.001	13.0	< 0.001	16.4	< 0.001
Growth × *Leafshed*	17.5	< 0.001	3.4	0.07	1.2	0.28
Growth × *Climate*	8.0	< 0.001	7.2	< 0.001	5.5	< 0.001
Leafshed × *Climate*	6.9	< 0.001	5.7	< 0.001	2.6	< 0.05

Table 2 Statistical significance of the factors *Growth*, *Leafshed* and *Climate*, and their bivariate interactions, in explaining the variability in monthly percentage nutrient remobilization ($\%Nr_{BR(month)}$, $\%Pr_{BR(month)}$, and $\%Kr_{BR(month)}$)

Three-way ANOVAS were conducted for N, P and K separately. *Growth* stages were categorized as either GROWTH (when reproductive and/or vegetative growth are carried out) or NO-GROWTH. *Leafshed* was also categorized as either LEAFSHED (when > 10% of the yearly maximum leaf shedding of each species was measured) or NO-LEAFSHED (< 10%). *Climate* is the suitability of the climatic conditions when the remobilization event occurs. (See the Materials and Methods section for more details.) Nutrient remobilization percentages were arcsin(sqrt(x)) transformed before the analyses.

Relationship between nutrient use in growth and nutrient remobilization

The amount of nutrients remobilized per gram of branch along a given growing season ($Nur_{BR(year)}$) directly correlated with the amount of nutrients accumulated in the growing tissues in that season ($Nug_{BR(year)}$) (Fig. 3). This relationship was similarly strong for N, P and K ($P < 0.001$). The relationship between the relative amount of nutrients accumulated in vegetative organs ($Nugveg_{BR(year)}$) and that of nutrients remobilized ($Nur_{BR(year)}$) was similar. These patterns were consistent when all marked branches were considered, and also when only variation within each species was evaluated (see dashed lines in Fig. 3), except for K in *Q. ilex*. The nutrient investments in reproductive growth ($Nugrep_{BR(year)}$), on the contrary, exhibited no direct relationship with $Nur_{BR(year)}$.

Discussion

Leaf shedding and NP remobilization peaked in mid-summer, while growth peaked in spring

A number of previous works have reported a tight chronological dependence of growth events and internal nutrient remobilization (Fife & Nambiar, 1982; Mark & Chapin, 1989; Nambiar & Fife, 1991; Jaeger & Monson, 1992; Oliveira *et al.*, 1996; Milla *et al.*, 2004). Here, however, although the seasonal distribution of N and P remobilization overlapped with part of the phenological activities, a remarkable delay between the peak in vegetative growth and flowering, and the peaks in N and P remobilization, was detected. In addition, K remobilization was nearly unrelated to growth phenology.

The time-lag between the peak in vegetative growth and N and P remobilization seems to be driven by the calendar of leaf abscission. Most of our species shed their leaves after *DVG*, mainly at the beginning of the summer. Nutrients reabsorbed during leaf senescence may account for a large proportion of the nutrients remobilized along the whole year (Cherbuy *et al.*, 2001). This contrasts with other evergreen species, where the remobilization of nutrients independently of leaf senescence is the major mechanism of N redistribution in the branches (Nambiar & Fife, 1991; Millard & Proe, 1993; Wendler *et al.*, 1995; Pasche *et al.*, 2002). By contrast, in the species of this study, leaf senescence appears to be the chief process leading to N and P remobilization. As pointed out in the Introduction section, spring–summer leaf senescence can be triggered by factors such as growth events or the arrival of summer drought in Mediterranean evergreens (Munné-Bosch & Alegre, 2004). Vegetative growth nutrient requirements had been fulfilled, either from soil uptake or from remobilization from roots or trunks, before the time when remobilization in the branches and leaf shedding peaked. The latter might therefore have been used to replenish nutrient reserves of older organs. However, leaf senescence might also have been promoted by the need of adjusting water transpiring surface at the beginning of the summer drought (Del Arco *et al.*, 1991).

An alternative explanation to the delay between growth and remobilization could lie in the seasonality of soil nutrient supply. Vegetative growth is more vigorous and frequently overlaps with flowering at mid-spring. In this period, soil nutrient availability and nutrient uptake by roots are high (Bonilla & Rodá, 1992; Serrasoles *et al.*, 1999), and could provide a large proportion of the nutrients required for growth. Both N and P remobilization peak sharply in mid-summer when soil supply is scarce. According to some authors, N remobilization predominates when environmental supply is low (Wendler *et al.*, 1995; Silla & Escudero, 2003). The formation of overwintering buds begins immediately after the elongation of vegetative shoots (Yuceer *et al.*, 2003), thus during the summer peak in N and P remobilization in the species of

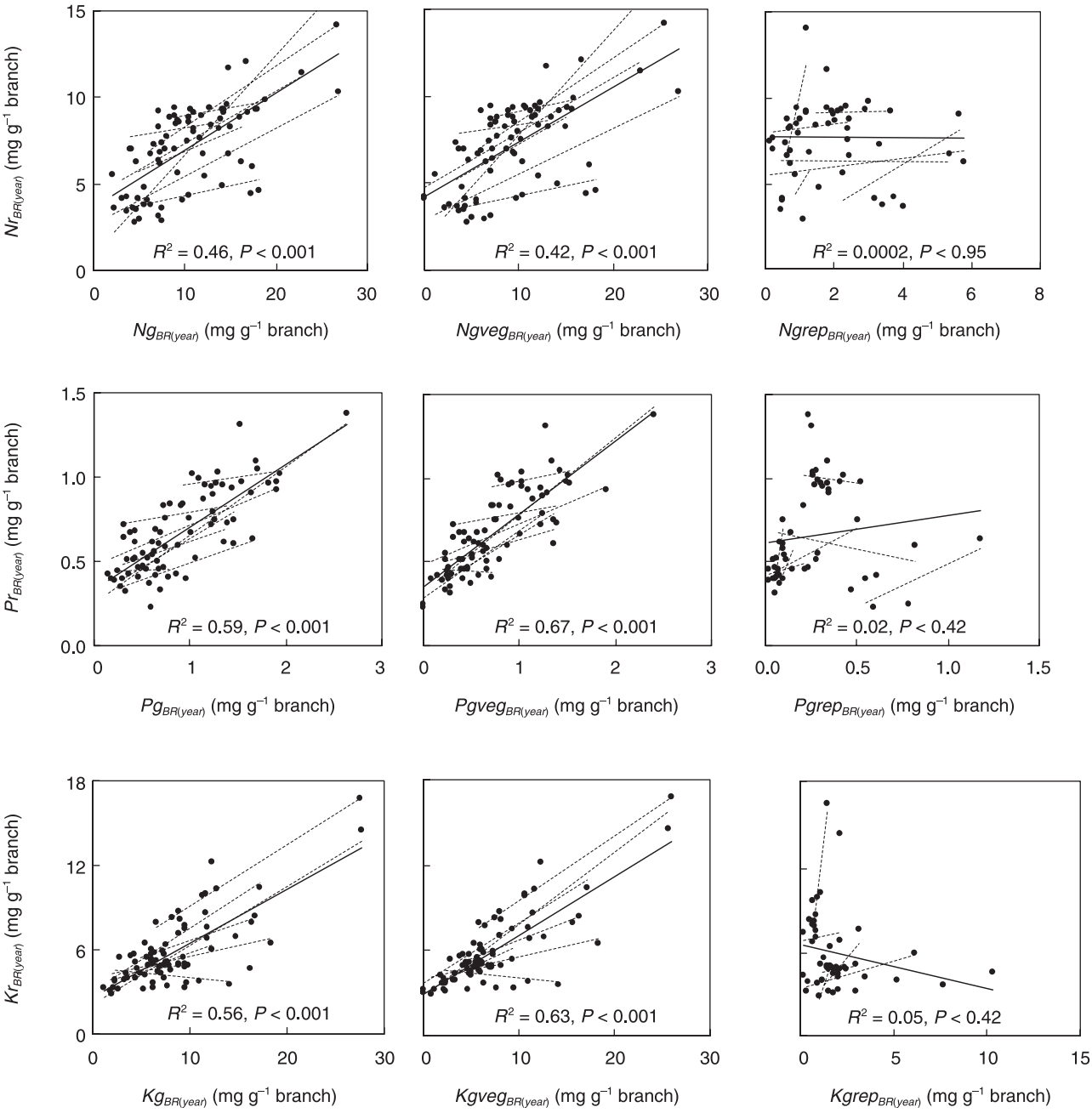

Fig. 3 Relationship between the amount of nutrients accumulated per gram of branch in the growing organs in a growing season (total mineralomass, $Ng_{BR(year)}$, $Pg_{BR(year)}$ and $Kg_{BR(year)}$; vegetative mineralomass, $Ngveg_{BR(year)}$, $Pgveg_{BR(year)}$ and $Kgveg_{BR(year)}$; reproductive mineralomass, $Ngrep_{BR(year)}$, $Pgrep_{BR(year)}$ and $Kgrep_{BR(year)}$) and the amount of nutrients remobilized from per gram of branch during the same period ($Nr_{BR(year)}$, $Pr_{BR(year)}$ and $Kr_{BR(year)}$). Dashed lines are adjusted linear regressions to the data of each particular species. Solid lines are the linear regression adjusted to the whole data set. R^2 values refer to the latter.

this study. In species with determinate growth, a large amount of N imported into the new organs occurs during bud growth (Millard, 1994). Furthermore, given that shoot extension slows down in summer, bud formation is likely to occur in a period of low external nutrient supply, and therefore requires more nutrients from internal remobilization. Therefore, although leaf senescence and massive N and P remobilization are unlikely to be triggered by the morphogenesis of overwintering buds, the latter probably benefited from the temporal coincidence of bud growth and N and P remobilization in the

stressful Mediterranean summer. We should note that belowground phenology was not monitored, and root growth might be an additional trigger of nutrient remobilization.

Nitrogen and P have some similar, but many distinct physiological functions. A large amount of N in green leaves is bound to the proteins of the Calvin cycle and thylakoids (Urban *et al.*, 2004), which are nearly absent in nonphotosynthetic (or poorly photosynthetic) reproductive organs. On the other hand, there is a great demand of P during flowering (Ashman & Baker, 1992; Niva *et al.*, 2003). In addition, P limits sexual reproduction in infertile environments (Nagy & Proctor, 1997; Brouwer *et al.*, 2001). Accordingly, N : P ratios were systematically higher in the vegetative than in the reproductive organs of the species of this study (data not shown), as has been found elsewhere (Ågren, 1988; Karlsson, 1994; Güsewell, 2004). Despite such differences, the seasonal patterns of N and P remobilization were very similar. This might be due to two facts. First, most biomass and nutrient pools in the branches were in vegetative organs (see Fig. 3). Therefore, despite P being more related to reproductive functions than N, P remobilization was more dependent on the growth of vegetative sinks than on reproductive, because of the smaller size of the latter. Second, most N and P remobilization probably came from leaf senescence in the branches of this study (see Fig. 2). During leaf senescence, N and P resorption often correlate positively, irrespective of the relative needs of each nutrient in the plant (Killingbeck, 1996).

The remobilization of K from the old parts of the branch peaked in periods of the year that differed greatly in phenological stage of the plants and climate. The main K remobilization event occurred in mid-summer, as did N and P, and could be due to K resorption before leaf shedding, or to the fulfilment of nutrient demands when soil availability is low, as explained earlier. However, K plays a crucial role in stabilizing cell pH and in regulating osmotic potential (Lansac *et al.*, 1994; Marschner *et al.*, 1997). In recently born organs, osmotic adjustment during the summer is required to endure drought conditions (Sanchez-Blanco *et al.*, 2002; Tognetti *et al.*, 2002). In addition, under moderate water stress, the accumulation of osmolytes in growing organs helps to increase cell turgor, thus facilitating growth (Mengel & Arneke, 1982; Boyer, 1988). Therefore, the remobilization of K in the summer could contribute to the maintenance of growth during this period. The depletion of K that occurs in mid-winter may also be related to the osmotic properties of this element. In mid- to late winter the renewal buds of most of our species start to swell. During this period evapotranspiration (Savé *et al.*, 1999) and temperatures are low. Given that K drives water to the meristems through osmotic water lifting (Zimmermann *et al.*, 2002), and also accumulates in tissues subjected to cold stress (Fernandez *et al.*, 2003), bud growth in winter could be facilitated by K remobilization from branch stores to the meristematic tips. Other studies also report that N is more related to growth requirements, and K to osmotic and carbohydrate transport functions (Proe *et al.*, 2000). However, in our calculations of $\%Nur_{BR(month)}$ we did not consider leaching as a potential mechanism of nutrient loss between sampling dates. Given that leaf K is easily leachable from the foliage (Wang *et al.*, 2003), this process could have accounted for some K losses during rainy months.

The amount of NPK remobilized per gram of branch was directly related to that accumulated in the growing vegetative organs along a season

If growth events were to exert a strong immediate effect on the remobilization of branch nutrient stores, this should be reflected in a close coupling between the outcome of both processes (nutrient pools in new organs, and remobilized nutrient pools from older organs) by the end of the growing season. In fact, branches that used more N, P or K for growth along the season also showed higher depletion of nutrient stores. Therefore, despite the relative asynchrony between growth and nutrient remobilization, the amount of reabsorbed nutrients correlated directly with the nutrient use for vegetative growth.

However, taking into account the time-lag between vegetative growth and remobilization, nutrients cannot be diverted directly from old to new vegetative biomass in the single branch, because remobilization occurs mainly after growth. What then is the rationale for this correlation? Several explanations can be suggested in this regard, though the selection of one of them would be speculative in the light of our data. First, the expansion of a new cohort of leaves probably changes the hormonal balances within the branch. As a consequence, old leaves should become a weaker sink for metabolites, and therefore are likely to begin senescence. Second, provided that growth events in long-lived perennials are highly dependent on internal remobilization (Aerts & Chapin, 2000), nutrient reserves other than old leaves (i.e. trunks or roots) are probably depleted of nutrients in early spring, which might be later replenished by the remobilization of nutrients from senescing leaves. Such complex seasonal remobilization dynamics have been reported previously in *Rhododendron* (Pasche *et al.*, 2002; Lamaze *et al.*, 2003). Moreover, in a number conifer species, a portion of the pool of nutrients taken up by roots and reabsorbed during the senescence of old needles in a given season is also temporarily stored in woody and needle tissues and invested later in the new vegetative growth during the following spring. This results in a close coupling between previous-year nutrient stores, and current-year nutrient use in growth, irrespective of current-year nutrient supply from the environment (Millard & Proe, 1993; Proe & Millard, 1994; Millard, 1996; Proe *et al.*, 2000). Third, the plant can supply water to a limited number of leaves during summer, and therefore the larger the newly expanded leaf area the higher the number of old leaves which have to be shed. This would imply that the measured relation between nutrient use and supply is an indirect

effect of the adjustment of leaf area when shoot growth has finished and summer drought is about to arrive. Previous work on the significance of nutrient remobilization for growth has rarely considered the chronology of both events. Our findings suggest that simple correlations between investments and supply can be incorrectly interpreted as direct source–sink functional relationships, if we lack phenological data.

Previous research on perennial or biennial herbs and grasses emphasized the dependence of reproductive events on internal N and P cycling (Heilmeier *et al*., 1986; Mark & Chapin, 1989; Jaeger & Monson, 1992; Bausenwein *et al*., 2001). In our study, although reproductive growth and N and P remobilization peaked in summer or at the end of spring, and therefore nutrient remobilization might be important to supply nutrients for reproductive events, the amount of nutrients remobilized was unrelated to the needs of reproductive organs. This was probably because of the comparatively lower resource use of reproductive growth (see Fig. 3) and, in accordance with the explanation in the preceding paragraph, to the fact that the development of reproductive structures does not display new leaf area which might trigger senescence-related nutrient remobilization.

In summary, in the branches of the studied species, N and P remobilization depend more on the chronology of leaf shedding than on growth events. Provided that the mineral nutrition of adult individuals of long-lived perennials is highly dependent on remobilization (Aerts & Chapin, 2000), the studied species should make use of organs other than the leafy branches which act as an intermediate storage site for the amounts of nutrients remobilized in summer and those used in the following spring growth event. The shortage of soil nutrient supply during summer drought could also promote nutrient remobilization in summer. Potassium remobilization appears to depend on factors such as demands for osmotic adjustment in climatically harsh periods of the year.

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