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Uptake, demand and internal cycling of nitrogen in saplings of Mediterranean *Quercus* species

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Abstract Nitrogen uptake, nitrogen demand and internal nitrogen cycling were studied to address the question of the importance of nutrient storage in *Quercus* species with contrasting leaf longevities. We carried out this study at the whole-plant level with young trees (2–4 years old) of three Mediterranean *Quercus* species: the evergreen *Q. ilex*, the marcescent/evergreen *Q. faginea*, and the deciduous *Q. pyrenaica*. Seasonal dynamics of nitrogen in all compartments of the plant were followed for 3 years. Nitrogen losses were measured through litter production, herbivory and fine root shedding. Nitrogen uptake was estimated using increments of nitrogen plant content plus accumulative nitrogen losses. Nitrogen uptake was limited to a few months during late winter and spring. Before budbreak, acquired nitrogen was stored in old-leaf cohorts of evergreen and woody compartments. After budbreak, *Quercus* species relied first on soil uptake and second on nitrogen retranslocation to supply new growth requirements. However, in most cases we found a high asynchrony between nitrogen demand by growing tissues and soil supply, which determined a strong nitrogen retranslocation up to 88.4% of the nitrogen demand throughout leaf expansion. Except for the first year after planting, the above- and underground woody fractions provided more nitrogen to the new tissues than the old leaf cohorts. Differences in the benefit of nitrogen withdrawn from senescent and old leaves were not found between species. We conclude that sink/source interaction strength was determined by differences between nitrogen demand and uptake, regulating internal nutrient cycling at the whole plant level.

Keywords Herbivory · Nutrient storage · Leaf expansion · Retranslocation · Resorption benefit

Introduction

Storage is a major function in most perennial species, especially in seasonal environments where it can play several roles. Storage is defined as ‘resources that build up in the plant and can be mobilized in the future to support biosynthesis’ (Chapin et al. 1990). Nutrient availability pulses characterize many seasonal environments that result in a strong asynchrony between uptake and demand (Bloom et al. 1985; Chapin et al. 1990). Storage allows plants to support leaf expansion and reproductive production during periods of relative low soil nutrient availability (Chapin and Shaver 1989; Jaeger and Monson 1992; Karlsson 1994). Fast seasonal growth in biennial and perennial species can be a critical component of competitive interactions that require a large mobilization of stored resources in a short span of time (Heilmeyer et al. 1986; Chapin et al. 1990). Species adapted to frequent disturbances invest in stored resources to support regrowth and guarantee persistence in the area after catastrophes (Bloom et al. 1985; Cannadell and Zedler 1995; Kruger and Reich 1997).

One controversial topic is the ecological significance of nutrient storage function of old leaf cohorts in relation to the fitness of evergreens in nutrient-poor habitats (Chapin 1980; Chabot and Hicks 1982; Escudero et al. 1992; Greenway et al. 1992; Karlsson 1994, 1995; Jonasson 1995a, 1995b; Eckstein et al. 1998; Aerts and Chapin 2000). Evergreen species accumulate several cohorts of leaves in the canopy, and invest a lower proportion of biomass in underground structures than deciduous species (Berendse and Elberse 1990; Gleeson and Tilman 1990; Aerts 1993; Reich 1998). Therefore, evergreens store a larger proportion of their total nutrient pool in leaves (Shaver and Chapin 1991; Shaver and Kummerov 1992). During leaf senescence, different chemical fractions break down allowing mobilization and re-use of nutrients. So, with similar resorption efficiencies between deciduous and evergreens (Aerts 1996; Killingbeck 1996), the potential contribution of nutrient resorption and mobilization of nutrients from old

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leaf cohorts towards new cohorts should be higher for evergreens than for deciduous species. Greater benefit from internal cycling would allow evergreens to depend less on soil uptake in nutrient poor sites. In addition, evergreen species can re-allocate nutrients directly from old and/or senescent leaves to the new growing leaf cohort (Reader 1978; Chabot and Hicks 1982; Chapin and Shaver 1989; Nambiar and Fife 1991; Karlsson 1994), saving carbon costs related to intermediate storage in roots and stems (Bloom et al. 1985).

However, different defoliation experiments have shown that there are not consistent differences in the benefit of nutrient resorption from old to new foliage between evergreen and deciduous species (Jonasson 1989, 1995a, 1995b; Greenway et al. 1992; Karlsson 1994; Eckstein et al. 1998). Response to defoliation experiments was unpredictable, irrespective of leaf life span, and despite a larger proportion of resource pool removed in evergreens than in deciduous trees (Eckstein et al. 1998). Two important issues are not considered in most of the works. First, the uptake capacity from soil is scarcely addressed. Nutrient uptake in low nutrient availability sites can be high enough to supply nutrients in species with low nutrient demand like evergreens compared to deciduous species with high nutrient demand (Jonasson 1989, 1995b). Second, nutrient storage in woody structures would supply a great proportion of nutrient demand throughout leaf expansion. Belowground structures are usually ignored despite large investments in root structures like rhizomes and lignotubers in many species (Cannadell and Zedler 1995; Aerts and Chapin 2000). Owing to the large biomass of woody tissues, small nutrient concentration changes would result in an important source of nutrient demand in deciduous and evergreen species.

The aim of our work was to study the relationships between nitrogen uptake, nitrogen demand and internal nitrogen cycling to address the question of the importance of nutrient storage in evergreen and deciduous species. We carried out this study with young trees (2–4 years old) of three Mediterranean *Quercus* species with different leaf life spans. Working with young trees allowed us to calculate the total pools of nitrogen in each plant compartment, and the nitrogen loss through different ways (litterfall, herbivory and fine root shedding). To our knowledge this is the first study that has combined nitrogen uptake, loss and demand patterns at whole plant level to study the importance of storage and retranslocation in species with contrasting leaf longevities.

Materials and methods

Species and study sites

Three *Quercus* species with contrasting leaf longevities were selected. *Q. ilex* L. subsp. *ballota* (Desf.) Samp. is a typical evergreen with a mean leaf longevity of 24–25 months. *Q. faginea* Lam. is a marcescent species with a mean leaf longevity of 8–9 months, but young plants like ours can retain leaves in the canopy until 14 months, behaving like evergreens. *Q. pyrenaica* Willd. is a

deciduous species with a mean leaf life span of 6–7 months. Relative dominance of *Quercus* species with different leaf longevities varies along nutrient, rainfall, and topographic gradients in central Spain. Two sites along a rainfall gradient were selected. The first site, the Miranda plot (40°55'N, 5°40'W, 790 m elevation), was planted with *Q. ilex* and *Q. faginea*. The site had a mean annual precipitation of 380 mm; soil had a pH of 7.66, and was very poor in nitrogen content (0.047%). The second site, the Cerralbo plot (41°00'N, 6°35'W, 720 m elevation), was planted with *Q. pyrenaica*. This site received a mean annual precipitation of 635 mm; soil had a pH of 7.75, and a nitrogen concentration of 0.056%. In February 1997, the Miranda plot was planted with 1,150 1-year-old seedlings of both *Q. ilex* and *Q. faginea*, and the Cerralbo plot with 2,150 1-year-old seedlings of *Q. pyrenaica*.

Plant sampling methods

Plants were sampled for 3 years, from March 1997 to January 2000. We collected samples every 7–15 days during the most active growing season (April–July), and every 1 or 2 months for the rest of the year. Between 8 and 12 seedlings of each species were randomly selected per sampling date. Each seedling was carefully excavated to at least 30 cm, to obtain the whole lignotuber and most of the fine and coarse plant root biomass.

Plants were fractionated into leaves, stems, lignotuber, fine roots (<2 mm diameter) and coarse roots (≥2 mm diameter). Leaves were classified into age cohorts and stems into year classes according to bud scars. Leaf area per age cohort was measured on fresh leaves, using a leaf area meter (Delta-T Mod. DIAS II, Delta-T Devices, UK). Insect damage to leaves was calculated tracing areas onto paper and estimating the missing area with the leaf area meter. Plant material was dried for 24 h at 80°C and weighed. Between four and six random replicates per fraction, species and sampling date were analysed to estimate nitrogen concentration using an autoanalyser (CE-Instruments NA-1100, ThermoQuest, Italy). The different stem year classes, except the new ones, were pooled before analysis.

Nitrogen pools, nitrogen uptake and loss

Nitrogen losses through three pathways were estimated: (1) leaf litter production, (2) herbivory by caterpillars and (3) fine root senescence. (1) To calculate leaf litter losses, we placed mesh litter traps in 20 randomly selected plants per species; the mesh was positioned around the whole plant to catch all the litter produced. Leaf and stem litter were harvested biweekly. Nitrogen losses were estimated multiplying leaf litter biomass values by leaf litter nitrogen concentration (mg g⁻¹). (2) We calculated nitrogen losses due to partial consumption of leaves by caterpillars using estimations of the missing area, leaf mass per area (LMA), and leaf nitrogen concentration between two sampling dates. (3) To estimate nitrogen losses due to root senescence we considered only the significant fall of fine root biomass that occurred throughout the summer. Nitrogen concentration of the fine roots during the lowest biomass harvest was used to estimate total nitrogen losses, considering nil nitrogen resorption from senescent roots (Nambiar 1987; Aerts 1989).

Nitrogen pools were estimated multiplying mean biomass values by mean nitrogen concentrations (mg g⁻¹). Cumulative nitrogen requirement was calculated by adding whole-plant mean nitrogen pool of each sampling date plus cumulative nitrogen losses up to that time. Nitrogen uptake from the soil was estimated as the increment of cumulative nitrogen requirement throughout the period considered. Nitrogen demand was defined as the amount of nitrogen required to meet the growth of the new leaf cohort and the new stems throughout leaf expansion.

Resorption efficiency (%) was calculated based on differences in nitrogen pool size per unit leaf area between fully expanded green and abscised leaves. We used resorption benefit (%) as the percentage of the total nitrogen demand supplied by nitrogen removed from abscised leaves. Nitrogen retranslocated pools based on old leaf and woody pool decreases were calculated only when

significant differences in nitrogen concentration (mg g^{-1} or mg cm^{-2}) were found between pre-budbreak and post-budbreak dates. We used retranslocation benefit (%) in a broad sense as the percentage of total nitrogen demand supplied by mobilization of nitrogen stored in woody and old leaf cohort fractions, including resorption benefit.

Statistical analysis

Cumulative demand and uptake patterns were fitted to different theoretical curves using least square regressions. Sigmoid model provided the best results. We used the following equation (Dixon 1976):

$$M(t) = \frac{P1}{1 + \exp\left[\left(\frac{2.2}{P3}\right)(P2 - t)\right]} \quad (1)$$

where $M(t)$ = cumulative nitrogen uptake or demand (in mg per plant), $P1$ = total annual nitrogen uptake or demand (in mg per plant), $P2$ = time of maximum rate of nitrogen uptake or demand (in days), $P3$ = time between 0.1 and 0.5 total nitrogen uptake or demand = time between 0.5 and 0.9 total nitrogen uptake or demand (in days), and t = time (in days). To analyse the relationship between nitrogen demand and uptake throughout leaf expansion, the uptake curve was set to zero just before budbreak. Maximum difference between demand and uptake curves was calculated and divided by cumulative demand at the end of leaf expansion to remove variation due to differences in plant nitrogen pool between years and species. A linear regression analysis was performed to test the relationship between retranslocation benefit and relative maximum difference between demand and uptake curves.

Nitrogen concentration and biomass data were not normally distributed (Shapiro- Wilk W test, $P < 0.01$). Previous to statistical analyses we used logarithmic (base 10) or arcsine transformation of the data, which generally normalized the data distribution and stabilized the error term variances (Sokal and Rohlf 1995). Data were further analysed using one-way and two-way ANOVA. If means were significantly different at the $P < 0.05$ level, a Tukey-Kramer HDS test was undertaken to look for differences between species and sampling periods. Analyses were performed using JMP statistical software (SAS Institute, USA).

Results

Nitrogen concentration trends

Before budbreak, the old leaf cohorts of *Q. ilex* and *Q. faginea* showed a significant increase in nitrogen concentration (Fig. 1, ANOVA: all old leaf cohorts $P < 0.01$), with the exception of 1999 for *Q. ilex*. Nitrogen concentration per unit leaf area followed a similar pattern and has not been shown. Woody fractions showed a similar pattern in the three species, with significant increments of nitrogen concentration throughout winter and early spring and before 1998 budbreak (Fig. 2, ANOVA: all stem and lignotuber fractions $P < 0.05$). In 1999 these increases were significant but smoother in *Q. faginea* and *Q. pyrenaica* (Fig. 2, ANOVA: stem and lignotuber fractions $P < 0.05$), and only significant in the stem fraction of *Q. ilex* (Fig. 2, ANOVA $P < 0.05$).

After budbreak, nitrogen concentrations in almost all woody fractions and old leaf cohorts dropped to their lowest values (Figs. 1, 2, Table 1). The lowest values of woody fractions were not statistically different between species (Fig. 2; ANOVAs not shown). Only 1999 *Q. ilex*

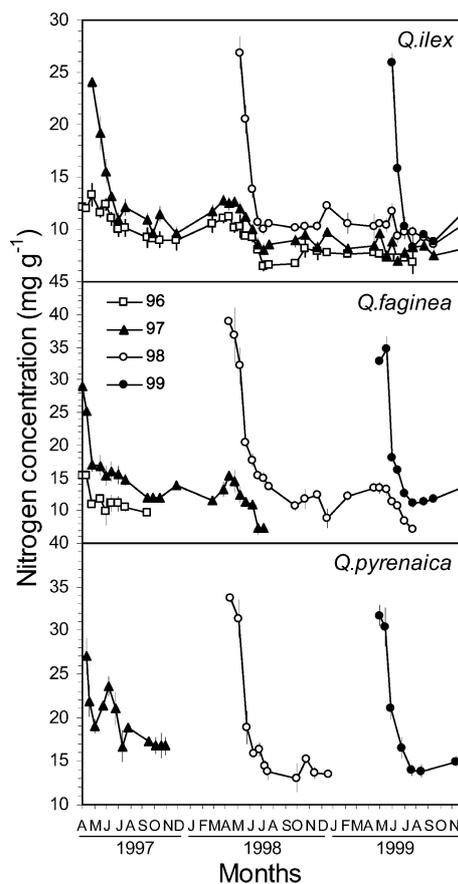


Fig. 1 Variation in nitrogen concentration (mean \pm SE) of the leaf cohorts in the three *Quercus* species over 3 years. $n=4-6$ for each sample occasion

showed significantly higher nitrogen concentration after budbreak in the old stem (ANOVA: $F_{2, 33}=5.89$, $P < 0.01$) and the lignotuber (ANOVA: $F_{2, 36}=8.52$, $P < 0.001$) than the other two species (Fig. 2). In addition, the old leaf cohorts of *Q. ilex* of the same age had higher nitrogen concentration in 1999 than in 1998 (ANOVA: 1-year old leaf cohort $F_{1, 22}=3.13$, $P < 0.01$, 2-year-old leaf cohort $F_{1, 21}=2.20$, $P < 0.05$). Nitrogen concentration was maintained low in most of the old tissues until replenishment in late winter and early spring except for the woody fractions of *Q. pyrenaica*, where a slight replenishment was observed throughout the autumn (Fig. 2). Nonsignificant changes were found in 1997 for the lignotubers of the three species, and in 1999 for all fractions of *Q. ilex* except the old woody stem (Table 1).

Nitrogen concentration of the fine roots of *Q. pyrenaica* and *Q. faginea* showed a seasonal pattern, with a peak in early spring and a subsequent drop to the lowest values in midsummer (Fig. 3). *Q. ilex* did not show this pattern except for the drop in nitrogen concentration in midsummer 1998 (Fig. 3). Nitrogen concentration had a significant positive relationship with the water content of fine roots in all three species (*Q. pyrenaica* $R^2=0.18$, $P < 0.01$; *Q. faginea* $R^2=0.19$, $P < 0.01$; *Q. ilex* $R^2=0.30$, $P < 0.005$).

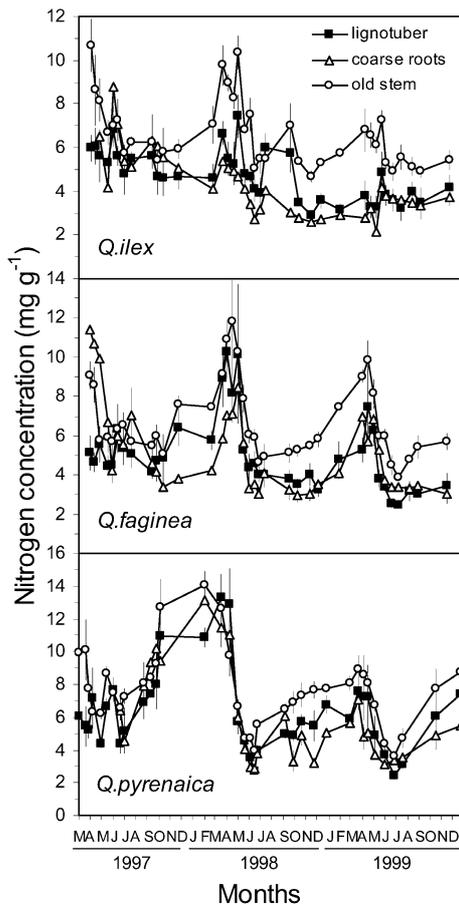


Fig. 2 Variation in nitrogen concentration (mean \pm SE) of the woody fractions in the three *Quercus* species over 3 years. $n=4-6$ for each sample occasion except for coarse roots (samples pooled in 1-2 subsamples)

Nitrogen internal cycling

Two phases could be clearly differentiated in the nitrogen pools of the old leaf cohorts (more than 1 year old) and the woody fractions: pre-budbreak storage and post-

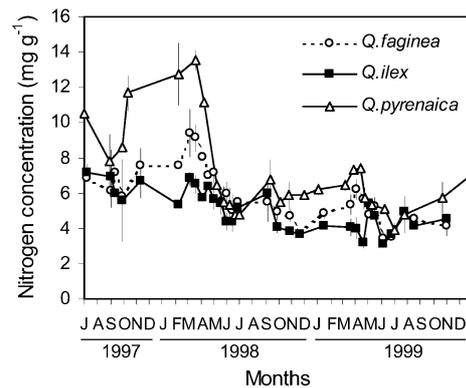


Fig. 3 Variation in nitrogen concentration (mean \pm SE) of the fine roots in the three *Quercus* species over 3 years. $n=4-6$ for each sample occasion

budbreak retranslocation to new tissues. Before budbreak, nitrogen pools of almost all leaf and woody fractions increased except those of *Q. ilex* in 1999 (Fig. 4). Increases in nitrogen pools were proportional to changes in nitrogen concentration since no significant increases in biomass were observed during late winter and early spring (data not shown).

Following budbreak, nitrogen was retranslocated to the new leaf cohort and stems. Strong nitrogen mobilization from stored reserves was found in all species and years, except in the 1997 *Q. pyrenaica* and 1999 *Q. ilex* growing seasons, where only minor drops associated with nitrogen retranslocation were observed (Fig. 4). Changes in nitrogen pool of old leaf cohorts after budbreak in *Q. faginea* and *Q. ilex* were mainly proportional to nitrogen concentration drops, since leaf senescence was mainly observed between 2 and 3 months after budbreak. Nitrogen retranslocation was a very fast process in all species, with a time span of no more than 2 weeks (Fig. 4). Retranslocation benefit was very high in most of the cases (except 1997 for *Q. pyrenaica* and 1999 for *Q. ilex*), supporting between 50.1 and 88.4% of the nitrogen demand during leaf expansion (Table 2). Except in 1997,

Table 1 Results of an analysis of variance testing for the differences of nitrogen concentrations of the plant fractions considered just before and after leaf budbreak. The data used in the ANOVA

analysis were pooled for the month just before budbreak and the month following budbreak

Fraction	F-test	<i>Q. ilex</i>			<i>Q. faginea</i>			<i>Q. pyrenaica</i>		
		1997	1998	1999	1997	1998	1999	1997	1998	1999
Lignotuber	P	0.261	0.012	0.138	0.930	<0.001	<0.001	0.650	<0.001	<0.001
	F	1.4	8.5	2.3	0.1	70.2	16.09	0.2	83.5	40.6
Old stem	P	<0.001	<0.001	0.021	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
	F	21.4	40.7	6.3	19.2	50.3	18.4	16.2	67.9	19.4
1996 leaf cohort	P	<0.001	<0.001	–	<0.001	–	–	–	–	–
	F	16.0	81.2	–	67.2	–	–	–	–	–
1997 leaf cohort	P	–	<0.001	0.665	–	<0.001	–	–	–	–
	F	–	101.9	0.2	–	92.3	–	–	–	–
1998 leaf cohort	P	–	–	0.731	–	–	<0.001	–	–	–
	F	–	–	0.1	–	–	3.95	–	–	–

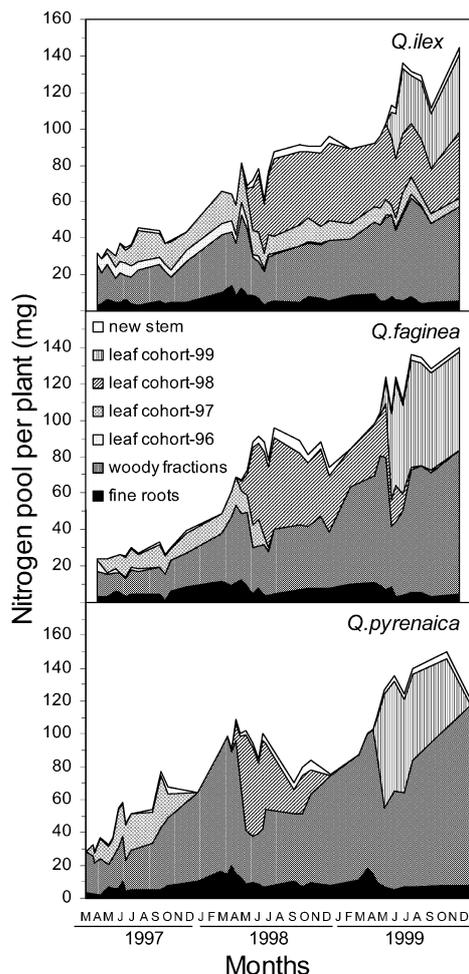


Fig. 4 Variation in nitrogen pool of all the fractions in the three *Quercus* species over 3 years. Data are mean pool (mg) per plant. Woody fractions (old stems, lignotuber and coarse roots) were pooled for simplicity

the woody fractions contributed most of the nitrogen retranslocation, ranging from 6.7 to 82.1% of the total nitrogen demand (Table 2).

Nitrogen losses and resorption

In the three species leaf fall was the main source of annual nitrogen loss, especially in the deciduous *Q. pyrenaica*, where it was up to 74% of total nitrogen loss (Table 3).

Table 2 Mean nitrogen retranslocation benefit (%) measured as the percentage of the total nitrogen demand by growing tissues supplied by the simultaneous mobilization of nitrogen stored in woody and old leaf cohort fractions for each year and each *Quercus* species. (*Qi*, *Q. ilex*; *Qf*, *Q. faginea*; *Qp*, *Q. pyrenaica*)

	1997			1998			1999		
	<i>Qi</i>	<i>Qf</i>	<i>Qp</i>	<i>Qi</i>	<i>Qf</i>	<i>Qp</i>	<i>Qi</i>	<i>Qf</i>	<i>Qp</i>
Old leaf cohorts	28.2	32.7	–	24.8	16.0	–	6.9	29.4	–
Woody fractions	26.0	18.3	6.7	39.4	34.1	82.1	10.1	59.0	56.5
Soil uptake	45.8	48.9	93.3	35.8	49.9	17.9	83.0	11.6	43.5

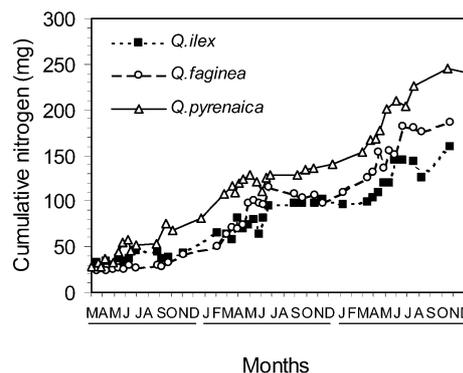


Fig. 5 Cumulative nitrogen requirements in the three *Quercus* species over 3 years. Cumulative requirement was estimated by considering nitrogen content plus nitrogen losses. Data are mean nitrogen pool (mg) per plant

On the other hand, the annual amount of nitrogen resorbed from senescing leaves was higher in *Q. pyrenaica* owing to greater litter production, and not to more efficient resorption (Table 3).

Nitrogen losses due to herbivory by caterpillars were significantly different between years and species but they affected only the expanding cohort (Table 3; two-way ANOVA: species $F_{2, 61}=21.5$, $P<0.001$, year; $F_{1, 61}=11.1$, $P<0.005$, species \times year; $F_{2, 61}=1.02$, $P=0.36$), not the old ones. Herbivore activity along the 1997 growing season was negligible for all species. For 1998 and 1999 the lowest nitrogen losses were found for *Q. ilex* (Table 3).

Estimates from fine root losses accounted for up to 43% and 31% of total nitrogen losses in *Q. ilex* and *Q. faginea*, respectively. In *Q. pyrenaica*, nitrogen losses from fine root shedding accounted for only 7.6% and 11% for 1998 and 1999, respectively (Table 3).

Nitrogen uptake, demand and retranslocation

The pattern of cumulative nitrogen requirement (estimated by adding nitrogen pools plus cumulative nitrogen losses) showed that nitrogen uptake from soil was limited to late winter and spring in all species in 1998 and 1999 (Fig. 5). Only *Q. pyrenaica* in the Cerralbo plot showed evidence of nitrogen uptake throughout spring and early summer 1997 after planting (Fig. 5).

Table 3 Nitrogen losses (mg per plant) through leaf senescence, herbivory and fine root shedding. Leaf nitrogen resorption efficiency (%), leaf litter production (g per plant) and leaf nitrogen resorption benefit (mg per plant) for each year and each *Quercus* species. Data represent means \pm SE, $n=20$. (Qi, *Q. ilex*; Qf, *Q. faginea*; Qp, *Q. pyrenaica*)

	1997			1998			1999		
	Qi	Qf	Qp	Qi	Qf	Qp	Qi	Qf	Qp
Leaf litter N losses (mg)	0.09 ^a \pm 0.07	0.81 ^b \pm 0.15	17.87 ^c \pm 2.31	3.09 ^{ab} \pm 0.39	7.81 ^b \pm 1.43	36.40 ^c \pm 4.38	3.66 ^{ab} \pm 0.61	17.53 ^b \pm 1.84	34.53 ^c \pm 3.69
Herbivory N losses (mg)	–	–	–	1.94 ^a \pm 0.43	7.23 ^b \pm 1.39	8.27 ^b \pm 1.36	0.77 ^a \pm 0.25	2.41 ^b \pm 0.68	6.90 ^c \pm 1.70
Fine root N losses (mg)	–	–	–	2.16	6.64	3.68	3.46	3.94	5.14
Total N losses (mg)	0.09	0.81	17.87	7.19	21.68	48.35	7.89	23.88	46.57
Leaf N resorption efficiency (%)	40.19 ^a \pm 1.14	56.57 ^b \pm 1.31	55.78 ^b \pm 2.06	43.99 ^{ab} \pm 2.73	54.47 ^b \pm 3.73	33.19 ^a \pm 3.08	38.12 ^{ab} \pm 1.42	48.98 ^b \pm 3.81	36.22 ^a \pm 2.43
Leaf litter production (g)	0.01 ^a \pm 0.01	0.19 ^b \pm 0.03	1.55 ^c \pm 0.19	0.44 ^a \pm 0.06	1.04 ^b \pm 0.14	3.03 ^c \pm 0.36	0.59 ^a \pm 0.10	1.80 ^b \pm 0.21	2.94 ^c \pm 0.30
Annual nitrogen resorbed from leaves (mg)	0.07 ^a \pm 0.06	0.94 ^b \pm 0.13	24.29 ^c \pm 3.18	2.66 ^a \pm 0.38	8.44 ^b \pm 0.99	12.69 ^b \pm 3.28	2.28 ^a \pm 0.49	11.37 ^b \pm 2.11	18.28 ^b \pm 2.52

Figure 6 shows the patterns of nitrogen uptake and nitrogen demand for new leaf cohort and stem growth during leaf expansion as well as the curves fitted to the data. Except for 1997, nitrogen demand was met faster in the deciduous *Q. pyrenaica* (6.9 ± 8.3 days, mean P3 parameter of sigmoid fit for 1998 and 1999) than in the evergreen *Q. ilex* (20.6 ± 11.2 days), while *Q. faginea* showed an intermediate rate of nitrogen accumulation in new tissues (12.4 ± 2.2 days). In all cases nitrogen demand by growing tissues was always higher than nitrogen supplied by soil absorption some time during leaf expansion (Fig. 6). However there were clear differences between the relative coupling of nitrogen demand and uptake between species and years. For example, uncoupling between both curves was the greatest in 1998 and 1999 for *Q. pyrenaica* (Fig. 6h, i), and the lowest in 1997 for *Q. pyrenaica*, and 1999 for *Q. ilex* (Fig. 6f, c).

Relative maximum difference between demand and uptake curves was statistically correlated with retranslocation benefit (Fig. 7, $R^2=0.79$, $P<0.005$). When the difference between demand and uptake was low the mobilization of stored nitrogen was equally small, and when nitrogen demand was only partially met by soil uptake, retranslocation of stored nitrogen supplied most of the leaf expansion demand (Fig. 7).

Discussion

Nitrogen uptake from soil was limited to late winter and especially to spring (60% and 83% of annual absorbed nitrogen in 1998 and 1999, respectively). Fine root nitrogen concentration clearly increased during spring in two of the three *Quercus* species suggesting a higher plant uptake (Eissenstat and Yanai 1997). Similarly, stomatal conductance and gas exchange were highest during spring in *Quercus* seedlings of the same species and plots (Mediavilla 2000), resulting in maximum transpiration, and likely, the incorporation of most nitrogen through mass flow (Eissenstat and Yanai 1997; Lambers et al. 1998; Berendse et al. 1999). Nitrogen content was correlated with water content in fine roots, showing that nutrient uptake is related to water availability in the soil. Water availability in the first 30 cm dropped dramatically throughout summer (data not shown), limiting water absorption and nitrogen acquisition (Nye and Tinker 1977; Chapin 1991; Evans and Ehleringer 1994). Seasonal patterns of plant uptake are characteristic of different ecosystems, where marked seasonal changes in temperature and/or precipitation result in cycles of nutrient availability (Campbell and Grime 1992; Jaeger and Monson 1992; Lodge et al. 1994; Bilbrough and Caldwell 1997; Jaeger et al. 1999). On the other hand, new growth requirements act as an important nitrogen sink, as shown in young *Fagus sylvatica* growing in experimental chambers where about 25% of total nitrogen was acquired during the first 6 weeks of leaf expansion after bud break (Dyckmans and Flessa 2002).

Fig. 6 Relationship between nitrogen demand and uptake along leaf expansion for *Quercus* species each year. Uptake curve was set to zero just before budbreak to compare differences between supply and demand. *Open circles* represent nitrogen demand (mean pool, mg per plant), *dashed line* represent best fit to nitrogen demand data points, *filled squares* represent nitrogen uptake (mean pool, mg per plant), and *solid line* represent best fit to nitrogen uptake data points

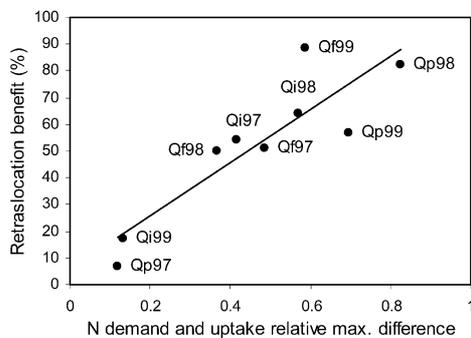
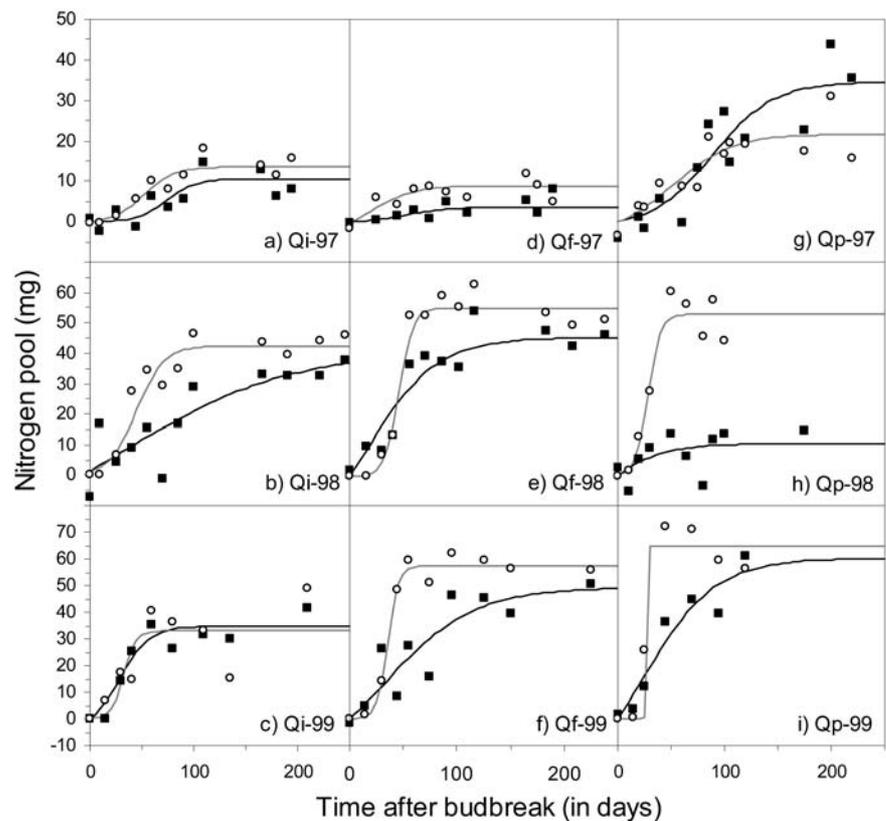


Fig. 7 Relationship between nitrogen benefit from retranslocation (%) and maximum relative differences between nitrogen demand and uptake curve fits. Points represent species and study years. (*Qi*, *Q. ilex*; *Qf*, *Q. faginea*; *Qp*, *Q. pyrenaica*)

In most of the cases analysed (except *Q. pyrenaica* 1997 and *Q. ilex* 1999), nitrogen uptake and nitrogen demand for new growth were partially asynchronous, with two stages clearly differentiated in the nitrogen internal dynamics: pre-budbreak storage and post-budbreak retranslocation. Before budbreak, acquired nitrogen was stored in the old-leaf cohorts of evergreens and the woody compartments of all species. Nitrogen concentration (%) increases were very similar in all plant fractions, and no preferences in storage allocation were observed. After budbreak, nitrogen pools of new tissues increased, primarily from nitrogen uptake and retranslocation. These

patterns have been found in plant species from extremely different communities, and are a consequence of a similar seasonal asynchrony between leaf demand and availability of nitrogen (Aerts 1989; Chapin and Shaver 1989; Jaeger and Monson 1992; Shaver and Kummerov 1992; Heilmeyer and Monson 1994; Karlsson 1994). Isotope labelling studies in growth chambers have also shown that leaf production in tree species relied mostly on previously acquired nitrogen resources, with a minor contribution of the newly assimilated nitrogen (Dyckmans et al. 2002).

In *Q. pyrenaica*, soil uptake during 1997 was high enough to support leaf expansion of the 2-year-old plants, but not in *Q. ilex* and *Q. faginea*, despite their lower nitrogen requirements (13.9 and 11.3 mg respectively vs 24.8 mg in *Q. pyrenaica*). Fine roots of *Q. pyrenaica* had higher a nitrogen concentration, greater specific root length (SRL), and lower tissue density, most likely allowing for a better growth response after planting (Silla 2001). Throughout 1998 and 1999, nitrogen retranslocation supported most of the demand of *Q. faginea* and *Q. pyrenaica*. However, in *Q. ilex*, the pattern was more variable with stored nitrogen supplying most of the demand in 1998 and soil uptake in 1999 (Table 3). Budbreak in *Q. ilex* occurred 3–4 weeks later than in *Q. faginea* and *Q. pyrenaica*, so leaf production of *Q. ilex* was more sensitive with regard to the length of a favourable growth period. Differences in nitrogen demand between 1998 and 1999 were due to greater leaf production in 1998 resulting from a moister spring (spring

precipitation was 130 mm in 1998 vs 72 mm in 1999) that caused the soil to dry more slowly (Silla 2001).

Our results showed that the contribution of retranslocation is correlated with the difference between demand and soil uptake. Thus, internal cycling dynamics are regulated by sink/source interactions, where the sink strength is determined by the uncoupling between nitrogen demand and soil supply, especially in fast-expanding species such as *Q. pyrenaica* and *Q. faginea*. Sink/source interactions are one of the main factors controlling nitrogen resorption from old leaf cohorts (Chapin and Moilanen 1991; Nambiar and Fife 1991), and probably all internal nutrient cycling at the whole plant level.

Nitrogen requirements of leaf expansion are met firstly by soil uptake and, secondly, by nitrogen retranslocation. When soil uptake was not able to supply enough nitrogen, plants mobilized stored nitrogen to meet the demand (all cases except 1997 and 1999 growing seasons of *Q. pyrenaica* and *Q. ilex*, respectively), but if soil supplies were high enough to support leaf expansion, the significance of retranslocation was small (1997 and 1999 growing seasons of *Q. pyrenaica* and *Q. ilex*, respectively). However, as our data show, the general trend was characterized by a strong uncoupling between supply and demand (Fig. 6), with leaf production relying mainly on retranslocation (Dyckmans et al. 2002).

Throughout late winter and spring 1999, we did not find replenishment in the woody fractions (except old stem) and old leaf cohorts of *Q. ilex*. As an alternative explanation, a lack of previous replenishment could prevent retranslocation to new tissues. However, significantly lower nitrogen content of old leaf cohorts of the same age in the previous year, and significantly lower nitrogen content of woody fractions after budbreak in *Q. faginea* and *Q. pyrenaica* suggest that old tissues of *Q. ilex* still had the potential to supply some nitrogen to new tissues in 1999.

Priorities on nitrogen source probably reflect the lower opportunity cost of soil uptake compared to formation/mobilization of stored nitrogen (Bloom et al. 1985; Chapin et al. 1990). In fact, if soil nitrate is assimilated in the leaves, the excess reducing power generated in the chloroplast can be used for nitrate reduction at a very low energetic cost (Layzell 1990; Poorter 1994). This hypothesis agrees with the results of Jonasson (1995a) who found that in defoliated individuals of the evergreen, *Rhododendron lapponicum*, soil uptake satisfied nitrogen demand without changes in woody pools.

The contribution of nitrogen from old leaf cohorts was between 6.9 and 28.2% and 15.9 and 32.7% of the total demand in *Q. ilex* and *Q. faginea*, respectively. Budbreak and leaf senescence were not synchronized in either species, and so nitrogen mobilized just after budbreak and nitrogen resorbed during leaf senescence were taken into account. The contribution of nitrogen resorption from senescent leaves in the deciduous species, *Q. pyrenaica*, was estimated at 45.9 and 19.6% of the nitrogen demand for 1998 and 1999, respectively. We did not find any clear differences between *Quercus* species concerning the

benefit of nitrogen withdrawn from old leaves, regardless of differences in leaf longevity. These results agree with the data reviewed by Karlsson (1994), and the lack of differential response to defoliation between deciduous and evergreen species found by Eckstein et al. (1998), despite the greater potential benefit to evergreens.

Although stored and recycled nitrogen from old leaf cohorts make important contributions to the growth of new tissues, soil uptake and retranslocation from above- and underground woody fractions supplied most of the nitrogen. Recycled nitrogen from old leaf cohorts contributed to new growth in evergreen and deciduous species (Reader 1978; Jonasson and Chapin 1985; May and Killingbeck 1992; Karlsson 1994; Eckstein et al. 1998). However, defoliation experiments with several species have shown that removal of nitrogen from old cohorts did not significantly affect the new growth (Jonasson 1989, 1995a; Greenway 1992; Eckstein et al. 1998). For example, in *Rhododendron lapponicum*, defoliation affected subsequent growth in some experiments (Karlsson 1994; Eckstein et al. 1998), but not in others (Jonasson 1989, 1995a). Differences related to phenological state and extension of defoliation have been proposed (Karlsson 1994, 1995), but Jonasson (1995a) pointed out that soil uptake compensated for nitrogen losses in his experiment. Our results clearly show, as Aerts and Chapin (2000) suggested, that it is not possible to make inferences about the importance of recycled and stored nitrogen considering only aboveground patterns of nitrogen loss from old leaves and nitrogen gain in new leaves. As we have demonstrated, retranslocation intensity from leaf and woody fractions is controlled by whole plant source-sink interactions. Therefore, we need to focus on the nutrient demand relative to soil uptake in order to understand the role of stored and recycled nutrients from leaf cohorts and woody fractions. This can be especially important in ecosystems like Mediterranean woodlands with great climatic variability between years, that affects leaf production, nitrogen demand and soil uptake.

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