

Nitrogen-use efficiency: trade-offs between N productivity and mean residence time at organ, plant and population levels

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Summary

1. Nitrogen-use efficiency (NUE_N) is often decomposed into the product of N productivity (A_N) and the mean residence time of N (MRT_N). Theory suggests a trade-off between both components, but direct experimental evidence is still scarce. A field study with young trees of the evergreen *Quercus ilex* and the marcescent-evergreen *Quercus faginea* was carried out to test this trade-off through analysis of plant traits at organ, whole-plant and population levels.

2. Specific leaf area (SLA) was the main trait positively related to A_N in *Q. faginea*. By contrast, greater litter production and consumption by caterpillars resulted in larger N losses and shorter MRT_N in *Q. faginea*. Early leaf senescence in *Q. faginea* produced leaf litter with high N concentration that contributed significantly to N loss. Moreover, *Q. ilex* had higher plant survivorship. The inverse relationship between leaf longevity and SLA is probably a key component of the trade-off between N losses and plant N productivity.

3. *Quercus faginea* had greater N uptake from soil, linked to its longer specific root length of fine roots and greater biomass allocation to underground tissues. Smaller N losses in *Q. ilex* compensated for its smaller N uptake and allowed a similar N balance at whole-plant level.

4. Our results support the hypothesis of a trade-off between A_N and MRT_N . *Quercus ilex* had a long MRT_N , while *Q. faginea* has a high A_N , and *vice versa*. The long MRT_N in *Q. ilex* involves not only reduced N loss through long intrinsic leaf life span, but also resistance to harsh environmental factors and defence against herbivores. This suggests that a long MRT_N is a potentially successful strategy in nutrient-poor environments.

Key-words: drought stress, herbivory, nitrogen loss, nitrogen uptake, plant strategies

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Introduction

Nitrogen availability limits plant growth in most non-tropical terrestrial ecosystems (Vitousek & Howarth 1991). After the seminal work of Vitousek (1982), the nutrient-use efficiency of N (NUE_N) emerged as a core concept for analysis of the relationship between carbon gain and the flux of nitrogen through plants. Berendse & Aerts (1987) redefined N-use efficiency as the product of N productivity (A_N , dry matter production per unit of N in the plant) and the mean residence time of N (MRT_N) in the plant. Berendse & Aerts (1987) also suggested that A_N and MRT_N would be inversely correlated owing to trade-offs between plant traits leading to high growth rates and those leading to reduced

N losses, restricting the variation in NUE_N values between species (Aerts 1990; Eckstein & Karlsson 1997). Thus nutrient-rich habitats will be dominated by fast-growing species (high A_N) with high rates of nutrient turnover (short MRT_N), while nutrient-poor habitats will show a predominance of slow-growing species (low A_N), with low nutrient-loss rates (long MRT_N).

Potentially fast-growing species exhibit a similar suite or 'syndrome' of leaf traits: high leaf N concentrations (mg g^{-1}); high specific leaf areas (SLA); short leaf life spans and high net photosynthetic capacities (Poorter & Remkes 1990; Garnier & Laurent 1994; Reich, Walter & Ellsworth 1997; Reich *et al.* 1999; Mediavilla & Escudero 2003). Additionally, slow-growing species have leaves with long life spans, low N concentrations (mg g^{-1}) and small SLA, thus reducing N losses and allowing long MRT (Escudero *et al.* 1992; Chapin, Autumn & Pugnaire 1993; Aerts 1995; Reich *et al.*

1999; Aerts & Chapin 2000; Wright, Westoby & Reich 2002). This evidence suggests that there should be a trade-off between plant traits that increase productivity and those that reduce nutrient-loss rates (Aerts 1995). Data concerning tissue density, tissue longevity, uptake rate and N concentrations of fine roots are still scarce, and the relationships reported between them are inconsistent (Schläpfer & Ryser 1996; Wright & Westoby 1999; Comas, Bouma & Eissenstat 2002; Espeleta & Donovan 2002). However, a trade-off between plant N uptake and N loss through fine roots would be expected if a higher nutrient uptake were correlated with larger N allocation and higher turnover rates in fine roots, considering N resorption from senescing roots to be generally negligible (Nambiar 1987; Aerts 1990; Gordon & Jackson 2000).

In contrast, direct experimental evidence of a trade-off between A_N and MRT_N at the plant level has been inconsistent (Garnier & Aronson 1998; Aerts & Chapin 2000). The predicted trade-off has been found when plant life-forms are compared, usually deciduous vs evergreen species (Aerts 1990; Eckstein & Karlsson 1997; Vázquez de Aldana & Berendse 1997; Eckstein & Karlsson 2001). Despite this, within a narrow range of leaf longevities (including within-species variation) the proposed trade-off has not been found (Aerts & de Caluwe 1994; Weih, Karlsson & Skre 1998; Eckstein & Karlsson 2001; Nakamura, Uemura & Yabe 2002; but see Yasumura *et al.* 2002). At least two reasons can be invoked to explain the absence of the trade-off under these circumstances (Eckstein, Karlsson & Weih 1999; Eckstein & Karlsson 2001): (1) the noise/response ratio is too high to detect patterns within the narrow range of traits considered; and (2) the trade-off does not necessarily exist because small changes in leaf life span may not necessarily have consequences for nutrient productivity.

Little information of the plant traits underlying this trade-off (or its absence) between the components of NUE_N is to be found in these studies, which limits analyses of the relationship between the indirect and direct experimental evidence (Garnier & Aronson 1998). Additionally, in most studies biotic factors (e.g. herbivory) and abiotic stresses (e.g. late frosts, severe water stress, etc.) were not considered, which would lead to an overestimation of MRT_N in fast-growing species characterized by more palatable (Coley 1988) and more fragile (high SLA) leaves (Reich *et al.* 1999; Wright *et al.* 2002).

In the present 2-year study we investigated the predictions of the trade-off posited by Berendse & Aerts (1987). We selected young trees of two Mediterranean *Quercus* species, with different leaf longevities, in a reforested site, allowing us to work with the whole plants under field conditions. We set out to answer three questions: (1) is there in fact a trade-off between A_N and MRT_N in the species selected? (2) are the plant traits that enhance A_N causally in conflict with those that promote long MRT_N ? and (3) how do biotic and

abiotic factors affect NUE_N components in relation to plant traits?

Materials and methods

SPECIES AND STUDY SITE

Two *Quercus* species with different leaf longevities were selected. *Quercus ilex* L. subsp. *ballota* (Desf.) Samp. is a typical evergreen with a mean leaf longevity of 20–22 months. *Quercus faginea* Lam. has a marcescent habit in adult trees (it retains the senescent leaves in the canopy during the winter), with a mean leaf longevity of 7 months, although young plants like ours (3–4 years old) can retain leaves until 14 months, behaving mainly as evergreens. Both tree species co-exist as codominant in woody stands, although in central Spain relative abundance usually varies along nutrient, rainfall and topographic gradients. In woodlands and savannahs (*dehesas*) *Q. ilex* dominates in habitats with nutrient-poor and shallow soils, while *Q. faginea* is predominant in habitats with deeper soils (García-Rodríguez 1991).

Our study site was a reforested old field located 12 km south of the city of Salamanca (40°55' N, 5°40' W, 790 m a.s.l.). The study area was dominated by *Quercus* woodlands, although it had been extensively deforested for agricultural purposes many years previously. The site has a mean annual precipitation of 380 mm; the soil has a pH of 7.66 and is very poor in N (0.047%).

PLANT SAMPLING PROCEDURE

In February 1997 an experimental plot of 1 ha was planted with 1150 1-year-old seedlings per species of *Q. ilex* and *Q. faginea*. From October 1997 to November 1999 we collected plants every 2 weeks during the most active growing season (April–July), and every 1 or 2 months over the rest of the year. Between eight and 12 seedlings of each species were collected randomly on each sampling date. Each seedling was carefully excavated to a depth of at least 30 cm in order to obtain most of the plant's fine root biomass. Seedlings were stored in plastic bags, carried in portable iceboxes to the laboratory, and kept frozen until processing.

Plants were divided into leaves, stems, lignotubers, fine roots (<2 mm diameter) and coarse roots (≥2 mm diameter). Leaves were sorted into annual cohorts, and stems into year classes according to bud scars. Leaf area was measured on fresh leaves using a leaf-area meter (Delta-T Mod. DIAS II, Delta-T Devices Ltd, Cambridge, UK). Insect damage to leaves was estimated by tracing areas onto paper and estimating the missing area with the leaf-area meter. Fine roots were washed with tap water except for one subsample per plant, which was carefully cleaned with a brush. In the subsamples the length of the fine roots was measured to the nearest millimetre, and the largest and smallest diameters of the ends of short fragments of fine roots

were measured with a digital caliper (Digimatic 500, Mitutoyo, Utsunomiya, Japan). The specific root length (SRL, length of fresh fine roots/dry biomass) and tissue density (dry biomass/volume of tissue in fresh fine roots) were calculated. The volume of fine roots was estimated, considering them to be cylinders with uneven bases. Plant material was dried for 48 h at 80 °C and weighed. Between four and six random replicates per fraction, species and sampling date were analysed to estimate N contents using an autoanalyser (CE-Instruments NA-1100, ThermoQuest, Milan, Italy).

CALCULATIONS

The leaf weight ratio (LWR, leaf dry biomass/whole-plant dry biomass) showed seasonal variations; here only the maximum values attained for each species during late spring and early summer are included. The mean fine-root N content per unit length (mg m^{-1}) was calculated as the product of the mean fine-root N concentration (mg g^{-1}) and the SRL (mg m^{-1}).

New leaf and wood production were estimated at the end of the growth season (September–October). However, in 1998 we added late summer/early autumn litter data to the standing new leaf biomass of *Q. faginea* because a strong summer drought stress led to early senescence of part of the new cohort of leaves. We calculated the secondary growth of old stems, coarse roots and lignotubers using data on the mean weight increase between the late and early growth season sampling dates. Fine-root production and mortality were estimated by summing statistically significant changes in the fine-root biomass between sampling periods. Although we underestimated some fine-root mortality due to the continuing birth and death of fine roots (López, Sabaté & Gracia 2001), most production and mortality probably occurred asynchronously owing to strong seasonal variations in soil water contents (Lamont 1995) (from $13.2\% \pm 0.6$ in May to $6.3\% \pm 0.8$ in July; data represent mean water contents at 30 cm depth \pm SE; Silla 2001). In addition, it is believed that more precise methods, such as the minirhizotron technique, underestimate production in the top 10 cm of soil (a depth critical in our young trees), compared with more classic techniques such as those used in the present work (López *et al.* 2001).

The leaf expansion rate was defined as the mean percentage of new leaf area expanding daily. It was calculated according to the expression:

$$\text{Leaf expansion rate} = 1/(t_1 - t_0) \times 100 \quad \text{eqn 1}$$

where t_1 is the date of completed leaf expansion, and t_0 the date of budbreak. We estimated t_0 as the date when 50% of the plants had reached budbreak.

Nitrogen losses were estimated on the basis of four different mechanisms: leaf litter production; herbivory by caterpillars; fine-root losses; and plant mortality. (1) To calculate leaf litter losses mesh litter traps were

placed around 20 randomly selected plants per species. The mesh was positioned around the whole plant to catch all the litter produced. Leaf and stem litter was harvested twice weekly. (2) Biomass losses due to caterpillar consumption were calculated using estimations of missing areas and leaf mass per unit areas (LMA) during the first month of leaf expansion, precisely when caterpillar damage and area losses were significant for both species (Silla 2001). Nitrogen losses were estimated on the basis of the missing leaf area and the mean leaf N content (mg cm^{-2}). (3) Fine-root mortality was estimated as described for fine-root production. The N content of fine roots during the minimum biomass harvest was used to calculate N losses through fine-root death, considering N resorption from senescent roots to be zero (Nambiar 1987; Aerts 1990; Gordon & Jackson 2000). (4) After planting, 80 individuals of each species were marked with flags to estimate relative mortality until the end of the sampling period. Nitrogen losses through whole-plant mortality were estimated by multiplying the number of plants that had died by mean N contents per plant at the time of death.

Nitrogen pools were estimated by multiplying mean biomass values by mean N concentrations. The cumulative N pool was calculated by adding the total mean N pool to cumulative N losses over time. Annual N uptake was estimated as the increase in the cumulative N pool during the study years (see Silla & Escudero 2003 for details).

The calculation of NUE components is based on the assumption that the plant is in a 'steady state' (nutrient uptake equals nutrient loss; Berendse & Aerts 1987; Garnier & Aronson 1998), which cannot be said of our trees. Accordingly, we calculated N productivity for shorter intervals, as proposed by Vázquez de Aldana & Berendse (1997), according to the following equation:

$$A_{N(t)} = (B_2 - B_1 + L)/[(N_{\text{pool}2} - N_{\text{pool}1})/2] \quad \text{eqn 2}$$

where B and N_{pool} are the plant biomass and N pool size, respectively, in two consecutive harvests, and L is the total biomass loss estimated between the two harvest as shown for N losses (see previously). Annual N productivity (A_N) was calculated by summing the estimates of $A_{N(t)}$ for six harvest periods (Vázquez de Aldana & Berendse 1997; Eckstein & Karlsson 2001): (1) January–April; (2) May; (3) June; (4) July; (5) August–September; (6) October–December. Leaf N productivity (LNP) was calculated in the same way, considering leaf N pool sizes instead of whole-plant N pool sizes. At population level, N productivity was calculated for each period by multiplying biomass production and N pools by population density.

At plant level the MRT_N is more difficult to calculate properly, even under steady-state conditions (Eckstein *et al.* 1999; Eckstein & Karlsson 2001). Here the MRT_N was calculated as the ratio between the average whole-plant N content and annual N losses. We started our study in the third year of growth (the second after

planting), so the plants were older than the mean longevity of the leaves, thereby avoiding the strong changes in LWR of the two first years for both species (Silla 2001). We also estimated MRT_N at population level. The population N pool was calculated by multiplying the average whole-plant N content by average plant density, and plant mortality was added to the plant-population N losses. As pointed out by Eckstein *et al.* (1999), the main flaw in this approach appears to be that the estimate obtained is only representative of the growth period of the study. However, this should not limit the meaningfulness of the MRT for the purposes of comparing plant strategies (Eckstein *et al.* 1999; Eckstein & Karlsson 2001). Additionally, understanding of variations in NUE_N components is probably more important during the initial stages of initial growth, when most mortality occurs, to explain the dominance or coexistence of species with characteristic ‘syndromes’ of traits.

Nitrogen-use efficiency (NUE_N) was calculated as the product of N productivity (A_N) and mean residence time (MRT_N) according to Berendse & Aerts (1987).

The data on biomass and N pools did not follow a normal distribution (Shapiro–Wilk W -test, $P < 0.01$), hence logarithmic (base 10) or arcsine transformations of the data were used before statistical analyses (Sokal & Rohlf 1995). This transformation normalized the data distribution and stabilized the variances in the error terms. The data were further analysed, mainly using two-way ANOVA, with species and study year as the two factors, testing for significant interactions between them. For simplicity only the differences between species and significant interactions are shown. Differences between years are shown where relevant. Analyses were performed using the JMP statistical software (SAS Institute, Cary, NC, USA).

Results

PLANT BIOMASS, LEAF AND FINE-ROOT TRAITS

At the end of each growing season, total biomass was not significantly different ($P > 0.05$) between the species (Fig. 1). However, after 3 years plant survival was around 90% in *Q. ilex*, and only 68.3% in *Q. faginea* (Fig. 2). Most plant mortality occurred in the summer of 1998, and was zero during 1999 (Fig. 2). The leaf-mass ratio (LMR) was twofold greater in *Q. ilex* (Table 1; $P < 0.001$). The significantly greater relative allocation to leaf biomass observed in *Q. ilex* was due to the accumulation of three leaf cohorts in each plant. By contrast, the higher SLA in *Q. faginea* led to a significantly larger leaf area per plant (Table 1; $P < 0.05$). With respect to the N concentrations per unit mass ($mg\ g^{-1}$) of the new fully expanded leaves, *Q. faginea* had significantly higher values than *Q. ilex* (Table 1; $P < 0.001$), although the N concentrations per unit area ($mg\ cm^{-2}$) showed the inverse pattern (Table 1;

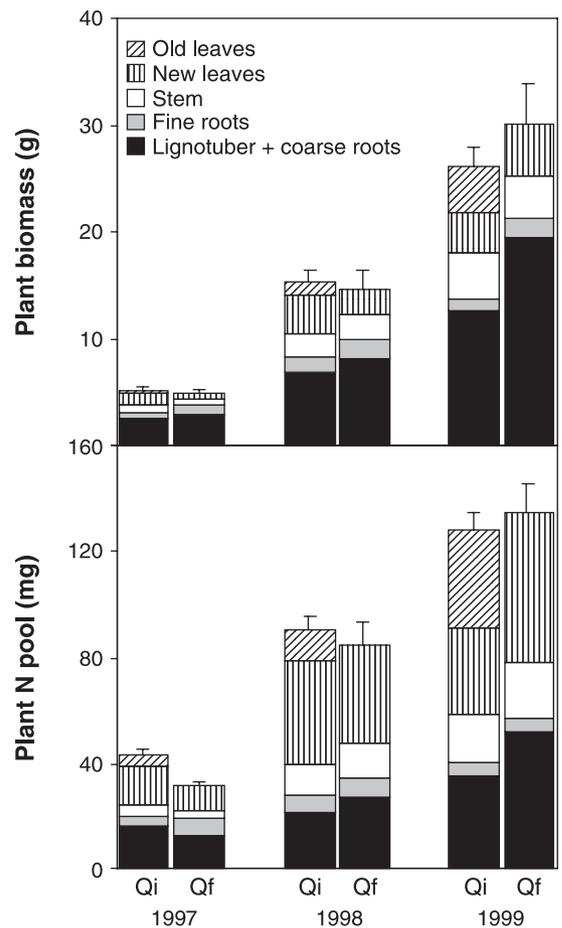


Fig. 1. Biomass (g per plant ± SE) and nitrogen pool (mg per plant ± SE) at the end of the growth season in different plant fractions of *Quercus ilex* (Qi) and *Quercus faginea* (Qf).

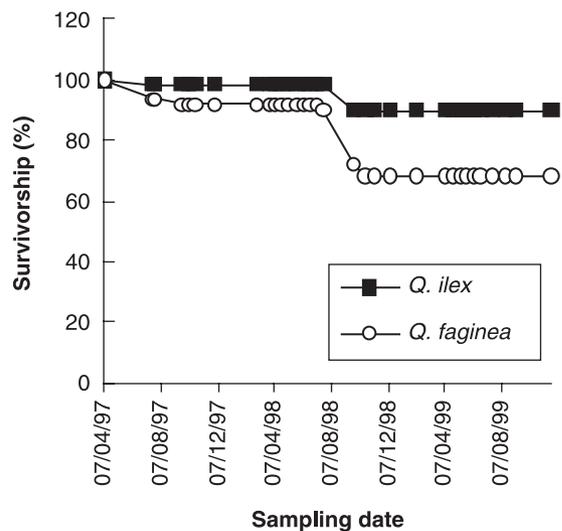


Fig. 2. Plant survivorship (%) of *Quercus ilex* (Qi) and *Quercus faginea* (Qf) during the study period ($n = 80$ trees per species).

$P < 0.001$). Thus *Q. ilex* allocated 54–57% of plant N to leaves, while *Q. faginea* allocated a significantly ($P < 0.001$) lower proportion, between 42 and 44% (Fig. 1) to this end.

Table 1. Leaf mass area, plant leaf area, fine-root biomass, and leaf and fine-root traits of *Quercus ilex* (Qi) and *Quercus faginea* (Qf) for the two study years

Plant trait	Year	Species	
		Qi	Qf
Leaf mass area (g g ⁻¹)	1998	0.317 ± 0.01 (16)	0.172 ± 0.01 (17)
	1999	0.318 ± 0.02 (16)	0.160 ± 0.011 (16)
Peak plant leaf area (cm ²)	1998	227.0 ± 16.3 (21)	320.7 ± 25.0 (28)
	1999	362.8 ± 37.7 (16)	457.3 ± 54.1 (16)
Peak fine root biomass (g)	1998	1.51 ± 0.10 (38)	1.77 ± 0.11 (39)
	1999	1.73 ± 0.15 (21)	2.24 ± 0.18 (21)
Specific leaf area (cm ² g ⁻¹)	1998	56.7 ± 0.99 (25)	91.4 ± 2.04 (16)
	1999	53.6 ± 1.16 (19)	94.9 ± 2.23 (24)
Daily leaf expansion rate (%)	1998	1.72	2.27
	1999	1.43	2.50
Leaf N (mg g ⁻¹) of the new cohort	1998	10.4 ± 0.19 (13)	12.2 ± 0.66 (11)
	1999	9.8 ± 0.40 (21)	12.3 ± 0.40 (20)
Leaf N (mg cm ⁻²) of the new cohort	1998	0.170 ± 0.006 (13)	0.151 ± 0.006 (11)
	1999	0.163 ± 0.006 (21)	0.139 ± 0.007 (20)
Specific root length (m g ⁻¹)	1998	2.45 ± 0.17 (35)	3.06 ± 0.26 (34)
	1999	2.32 ± 0.12 (17)	3.10 ± 0.20 (16)
Fine root tissue density (g cm ⁻³)	1998	0.217 ± 0.03 (35)	0.194 ± 0.04 (34)
	1999	0.200 ± 0.02 (17)	0.186 ± 0.02 (16)
Fine root N content at peak biomass (mg g ⁻¹)	1998	6.13 ± 0.25 (17)	8.56 ± 0.39 (19)
	1999	4.29 ± 0.22 (19)	5.53 ± 0.21 (20)
N content per fine root length (mg m ⁻¹)	1998	2.50	2.79
	1999	1.85	1.78

Data represent means ± SE; *n* is given in parentheses.

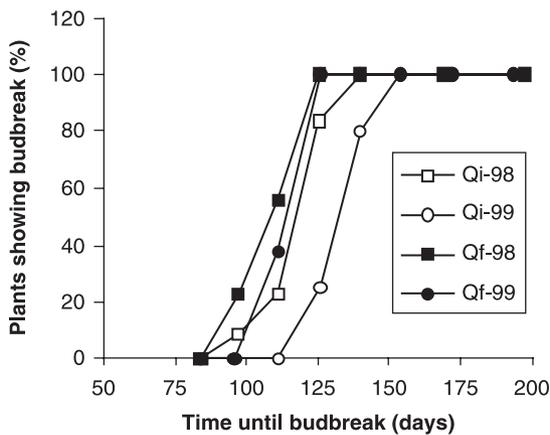


Fig. 3. Percentage of plants showing budbreak of the new leaf cohort in *Quercus ilex* (Qi) and *Quercus faginea* (Qf) during the two study years. Time until budbreak was set to zero at 1 January (*n* = 12 for each species and sampling date).

Leaf budbreak occurred up to 3 weeks earlier in *Q. faginea* than in *Q. ilex* (Fig. 3). The budbreak phenology of *Q. ilex* in 1999 had a time lag of 2–3 weeks with respect to 1998 (Fig. 3). The rate of daily leaf expansion of the new cohort was faster in *Q. faginea* than in *Q. ilex* (Table 1). Combining an earlier leaf phenology with faster leaf expansion, *Q. faginea* showed a fully developed canopy around the second half of June, while in *Q. ilex* the new leaf area was developed 1 month later in both study years (Fig. 4). In 1998

Q. faginea lost almost 50% of its new leaf area after summer (Fig. 4) due to an unusually early leaf senescence associated with a strong drought (during July and August precipitation was 1 mm in 1998 compared with 12.4 mm in 1999).

A peak in fine-root biomass was attained by both species at the end of spring (seasonal variation in biomass not shown), and was greater in *Q. faginea* than in *Q. ilex* (Table 1; $P < 0.005$). The fine roots of *Q. faginea* exhibited a significantly lower tissue density (Table 1; $P < 0.01$) and a higher SRL (Table 1; $P < 0.01$) than those of *Q. ilex*. At the fine-root biomass peak, N concentrations (mg g⁻¹) were higher in the fine roots of *Q. faginea* than in *Q. ilex* (Table 1; $P < 0.001$), although there were significant within-species differences between years ($P < 0.001$). Owing to differences in SRL and fine-root N concentrations, the N content per fine-root length (mg m⁻¹) was similar for both species (Table 1).

Both species had similar annual leaf productions in both study years (Table 2; $P > 0.10$). In addition, stem production per year was similar for both species (Table 2). However, total underground productivities were 34 and 100% greater in *Q. faginea* for 1998 and 1999, respectively (Table 2). As a result, the total annual net primary productivity (aNNP) was 17.0 and 57.7% greater in *Q. faginea* for 1998 and 1999, respectively (Table 2). The differences in aNNP were lower at population level than at individual plant level (Table 2) due to the effects of higher mortality on plant density in *Q. faginea*.

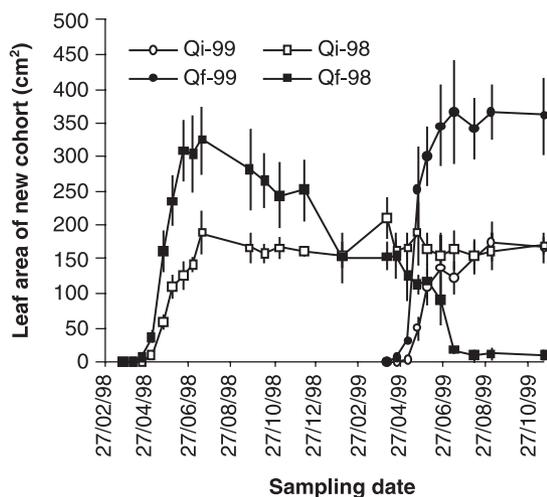


Fig. 4. Leaf area per plant of the new cohort ($\text{cm}^2 \pm \text{SE}$) in *Quercus ilex* (Qi) and *Quercus faginea* (Qf) throughout 1998 and 1999 ($n = 8\text{--}12$ for each species and sampling date).

NITROGEN LOSSES

Losses of N due to leaf abscission were 43–47 and 36–59% of the total N losses in *Q. ilex* and *Q. faginea*, respectively (Table 3). Nitrogen losses in leaf litter were higher in *Q. faginea* than in *Q. ilex* due to between-species differences in litter production (Table 3; $P < 0.001$) and in mean leaf litter N concentrations (mg g^{-1}) between the species (Fig. 5; $P < 0.001$). Leaf litter N contents (mg g^{-1}) were significantly greater in *Q. faginea* for the period between September 1998 and March 1999 (Tukey–Kramer HDS, $P < 0.05$). During this period most of the leaf litter shed was made up of leaves that had died during the summer drought of 1998; these remained dry on the tree and were shed gradually during the following autumn–winter (Fig. 4). During the rest of the study period there were no significant differences in litter N concentrations between species (Fig. 5).

Nitrogen losses due to herbivory by caterpillars were significantly different between years (Table 3; $P < 0.005$)

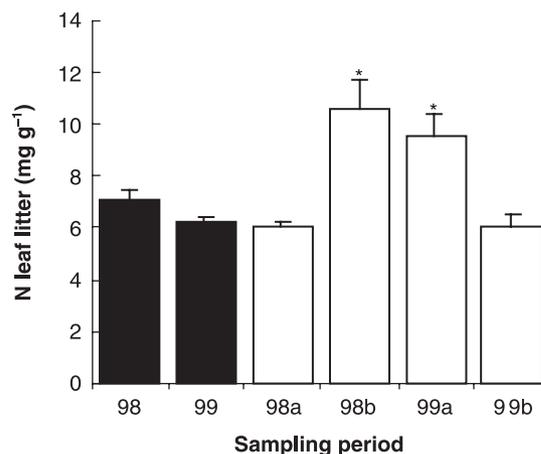


Fig. 5. Leaf litter nitrogen concentration ($\text{mg g}^{-1} \pm \text{SE}$) of *Quercus ilex* (solid bars) and *Quercus faginea* (open bars) for each sampling period. Values of *Q. ilex* are annual means; for *Q. faginea* two sampling periods per year are shown (98a: January–August; 98b: September–December; 99a: January–March; 99b: April–November). Asterisks indicate significant differences ($P < 0.05$) between species and sampling periods.

and species (Table 3; $P < 0.001$). *Quercus faginea* lost three to four times more N than *Q. ilex*, mainly due to differences in the leaf area eaten by caterpillars (Table 3; $P < 0.001$). The N contents (mg g^{-1}) of young leaves of both species were similar at the time of caterpillar activity (Table 3; $P > 0.10$).

Estimates from fine-root losses accounted for up to 44 and 31% of total N losses in *Q. ilex* and *Q. faginea*, respectively. In *Q. faginea* higher fine-root N concentrations for both years (Table 3; $P < 0.01$) and larger biomass losses in 1998 led, on average, to larger N losses (Table 3). Biomass losses were up to 2.4-fold greater in *Q. faginea* than in *Q. ilex* during the dry summer of 1998, but were very similar between both species during 1999. For both species the N concentrations in fine roots were significantly lower ($P < 0.001$) in mid-summer (at the time of minimum fine-root biomass harvest, data not shown) with respect to mid-spring (at the time of maximum fine-root biomass harvest, Table 1).

Table 2. Means \pm SE for leaf, stem, root, whole-plant productivity and population productivity of *Quercus ilex* (Qi) and *Quercus faginea* (Qf) plants for the two study years

Parameter	1998		1999	
	Qi	Qf	Qi	Qf
Plant productivity ($\text{g year}^{-1} \text{ plant}^{-1}$)				
New leaf biomass	3.79 ± 0.35	3.41 ± 0.41	3.66 ± 0.36	4.43 ± 0.35
Primary stem growth	0.58 ± 0.06	0.81 ± 0.08	0.80 ± 0.17	0.64 ± 0.24
Secondary stem growth	0.76	0.92	0.96	1.16
Lignotuber + secondary coarse root growth	3.77	5.08	4.82	9.80
Fine root increment	1.19	1.58	0.60	1.06
Plant annual net primary productivity	10.09	11.81	10.84	17.09
Population productivity ($\text{kg year}^{-1} \text{ ha}^{-1}$)				
Population annual net primary productivity	11.01	11.19	11.22	13.43

Table 3. Means \pm SE for biomass, leaf area and N losses, and N concentration of consumed leaves and fine-roots for *Quercus ilex* (Qi) and *Quercus faginea* (Qf) through leaf litter production, herbivory, fine-root senescence and plant mortality

Level	Parameter	1998		1999	
		Qi	Qf	Qi	Qf
Leaf litter	Biomass losses (g)	0.44 \pm 0.06	1.00 \pm 0.14	0.59 \pm 0.10	2.38 \pm 0.23
	N losses (mg)	3.09 \pm 0.39	7.81 \pm 1.43	3.66 \pm 0.61	17.53 \pm 1.84
Herbivory	Area lost (cm ²)	9.3 \pm 1.88	35.0 \pm 6.14	4.1 \pm 1.59	15.0 \pm 5.06
	N leaf (mg cm ⁻²)	20.9 \pm 0.9	20.6 \pm 0.8	18.7 \pm 1.2	16.0 \pm 1.0
	N losses (mg)	1.94 \pm 0.39	7.23 \pm 1.27	0.77 \pm 0.30	2.41 \pm 0.81
Fine roots	Biomass losses (g)	0.50	1.26	1.13	1.15
	Fine root N (mg g ⁻¹)	4.36 \pm 0.27	5.26 \pm 0.29	3.06 \pm 0.22	3.44 \pm 0.07
	N losses (mg)	2.16	6.64	3.46	3.94
Total N losses per plant (mg)		7.19	21.68	7.89	23.88
Plant N losses (g ha ⁻¹)*		7.44	17.04	8.17	18.77
Plant mortality	Mortality (plants ha ⁻¹)	95.8	268.3	0	0
	Plant N pool (g)	0.072	0.069	0.111	0.109
	N losses (g ha ⁻¹)	6.92	18.63	0	0
Total population N losses (g ha ⁻¹)		14.36	35.67	8.17	18.77

*Plant N losses (g ha⁻¹) include N losses at population level from leaf litter, herbivory and fine roots. Fine-root N concentrations are shown at time of minimum fine-root biomass.

Nitrogen losses due to plant mortality were almost three times higher in *Q. faginea* than in *Q. ilex* during 1998, but were negligible for both species during 1999 (Table 3). During 1998 N losses due to mortality were comparable to N losses through herbivory, leaf senescence and fine-root shedding for both species (Table 3).

WHOLE-PLANT N POOLS AND N UPTAKE

We found significant differences in whole-plant N pools between species (Fig. 1; $P < 0.01$), although the interaction between species and years was also significant (Fig. 1b; $P < 0.05$). The significant interaction was due to the fact that the increase in N between successive years was proportionally greater for *Q. faginea*, suggesting a higher N uptake in *Q. faginea* than in *Q. ilex* over the study period (Fig. 1). The estimates of mean annual N uptake from the soil (increase in plant N pools plus annual N losses) for *Q. ilex* were 57.4 and 45.0 mg per plant, but amounted to 75.4 and 67.6 mg in 1998 and 1999, respectively, for *Q. faginea*.

Total N pools were higher in *Q. ilex* at population level for both years (*Q. ilex*, 78.8 and 115.2 g ha⁻¹; *Q. faginea*, 64.2 and 85.9 g ha⁻¹, for 1998 and 1999, respectively), mainly due to the greater mortality of *Q. faginea* (Fig. 2) and the subsequent lower density.

NITROGEN-USE EFFICIENCY

In *Q. faginea*, N productivity (A_N) was 24 and 57% higher than in *Q. ilex*, and LNP was 43 and 86% greater for 1998 and 1999, respectively (Table 4). The differences in A_N were due to a higher aNNP in *Q. faginea*, as plant N pools were similar between species (when we considered only the 1998 and 1999 plant N pools the differences were not significant, $P > 0.10$). On the other hand, the larger differences in LNP were due to

Table 4. Means for N productivity (A_N), leaf N productivity (LNP), mean residence time of N (MRT_N), N-use efficiency (NUE_N) of *Quercus ilex* (Qi) and *Quercus faginea* (Qf) trees for the two study years at plant and population levels

Level	Year	Qi	Qf
Plant			
A_N (g g ⁻¹ N year ⁻¹)	1998	146.1	180.5
	1999	100.9	158.4
LNP (g g ⁻¹ N year ⁻¹)	1998	284.4	406.4
	1999	192.2	356.6
MRT _N (years)	1998	10.05	3.20
	1999	14.11	4.58
NUE _N (g g ⁻¹ N)	1998	1468.6	578.2
	1999	1422.7	725.0
Population			
A_N (g g ⁻¹ N year ⁻¹)	1998	146.3	179.3
	1999	100.9	158.4
MRT _N (years)	1998	5.24	1.54
	1999	14.11	4.58
NUE _N (g g ⁻¹ N)	1998	766.0	277.5
	1999	1422.7	725.0

a significantly lower N allocation to the canopy in *Q. faginea* (Fig. 1). In *Q. ilex* A_N and LNP dropped to around 31–32% in 1999 with respect to 1998, but only to around 12% in *Q. faginea*. Both species had almost the same N productivity at plant and population level (Table 4), perhaps because most biomass production occurred before the summer mortality of 1998.

At individual plant level, *Q. ilex* had an approximately threefold longer mean residence time (MRT_N) than *Q. faginea* (Table 4). Within species, the slightly longer MRT_N values found for 1999 (Table 4) were due to significantly greater N plant pools for 1999 ($P < 0.01$), but similar N losses for both years (Table 3). At population level, both species has shorter MRT_N in 1998 than for 1999 (Table 4) owing to differences in plant

mortality between the years. For 1998, the combination of larger individual N losses and greater plant mortality in *Q. faginea* resulted in larger interspecific differences in MRT_N at population level than at individual plant level (Table 4).

NUE_N was around 2–2.5 times higher in *Q. ilex* than in *Q. faginea* for both study years (Table 4). The similar NUE_N values in *Q. ilex* for 1998 and 1999 were due to compensatory changes in A_N and MRT_N . For *Q. faginea*, NUE_N was around 25% higher in 1999, mainly due to differences in MRT_N (Table 4). At population level, between-species NUE_N was almost threefold higher in *Q. ilex* during 1998 (Table 4). Between years, in 1998 the NUE_N was around half the value seen for 1999 for both species (Table 4).

Discussion

LEAF AND CANOPY TRADE-OFFS

Our results suggest that SLA was the main plant trait promoting greater A_N and LNP in *Q. faginea*. In young trees, like ours, *Q. faginea* had greater net leaf photosynthesis per unit area than *Q. ilex*, despite the lower N concentration per unit area (Mediavilla 2000), probably due to increased competition for CO_2 in the thicker mesophyll and a lower proportion of leaf N in the photosynthetic machinery of the low SLA leaves of *Q. ilex* (Mediavilla, Escudero & Heilmeyer 2001). Scaled up to whole-plant level, the high SLA in *Q. faginea* afforded a greater leaf area of the new cohort (Fig. 4), and a logically greater A_N (owing to similar plant N pools in both species) and LNP (owing to lower N allocation to leaves in *Q. faginea*). Old cohorts of *Q. ilex* showed low intrinsic net photosynthesis, affording a minor contribution to the whole-plant carbon balance (Mediavilla & Escudero 2003). Two additional traits promoted biomass production and consequently A_N : budbreak phenology and leaf expansion rate. Earlier budbreak and faster leaf expansion were combined in *Q. faginea* to produce full development of the new leaf cohort in mid-spring, around 1 month earlier than in *Q. ilex*. Due to summer water limitation, phenology is a candidate as a key trait in Mediterranean ecosystems to increase the carbon budget and A_N : phenology limits the period of foliage expansion in *Quercus* spp. (Pereira & Chaves 1993), and spring net photosynthesis is twofold that of summer net photosynthesis in the new leaf cohorts of *Q. ilex* and *Q. faginea* (Mediavilla 2000). The delayed phenology that occurred in *Q. ilex* in 1999 resulted in the incomplete development of the new cohort until mid-summer, precisely when the soil water content started to drop; this probably explains the dramatic drop of A_N and LNP in 1999 with respect to 1998.

The following leaf and canopy traits of *Q. ilex* allowed lower N losses and promoted longer MRT_N than in *Q. faginea*. (1) The long leaf life span prolonged MRT_N by increasing the retention time of N in

the canopy and reducing N leaf-litter losses (Escudero *et al.* 1992; Aerts 1995; Eckstein *et al.* 1999). (2) Leaf litter N concentrations were higher for *Q. faginea* from September 1998 until April 1999, although they were not significantly different from those of *Q. ilex* for the rest of the study periods. The dry summer of 1998 resulted in the cavitation of some terminal twigs in most *Q. faginea* plants, the loss of half of the new leaf area (Fig. 3), and a mortality of 32% in *Q. faginea* trees (Fig. 1). Cavitation has often been observed in temperate and Mediterranean *Quercus* species (Lo Gullo & Salleo 1993; Martínez-Vilalta *et al.* 2002). The vessels in the xylem of *Q. ilex* are smaller than those seen in *Q. faginea* (Villar *et al.* 1997), which allows resistance to cavitation to increase under drought stress (Martínez-Vilalta *et al.* 2002; Tyree 2003). Cavitation results in embolism, disrupting the flow of water (Tyree 1999), and to the abrupt death of leaves, probably affecting the resorption process in *Q. faginea*, as observed in *Q. ilex* (Escarré *et al.* 1999). (3) Caterpillars consumed larger leaf areas of *Q. faginea*, resulting in greater N losses than in *Q. ilex*, as occurs in mixed stands of adult trees (Pascual, Pérez & Peris 1994). In both species, caterpillar consumption was concentrated in young leaves with high N concentrations and a high SLA, the most attractive for herbivores (Hartley & Jones 1997). At the time of caterpillar activity, neither species had significant differences in leaf N concentrations due to differences in leaf phenology, because the leaves of *Q. faginea* exhibited a more advanced ontogenic stage and greater N dilution. However, almost the whole canopy of *Q. faginea* was formed by young and attractive foliage, while for *Q. ilex* most of the foliage was more than 1 year old, and it combined low SLA and N concentrations with hard spines and high lignin and phenol concentrations (Massei, Hartley & Bacon 2000). All these traits are effective in deterring and reducing the impact of insect herbivores within the canopy (Coley 1988; Hartley & Jones 1997; Zamora, Hódar & Gómez 1999).

At leaf level, our data support the hypothesis of a genotype-based trade-off between A_N and MRT_N (Berendse & Aerts 1987) due to the interrelationships between leaf life span and several different traits and processes. The inverse relationship between leaf longevity and SLA or tissue density is probably a key component of this trade-off (Schläpfer & Ryser 1996; Reich 1998; Reich *et al.* 1997, 1999; Aerts & Chapin 2000; Wright *et al.* 2002). Long leaf longevity requires a huge allocation to structural components, which provides physical resistance to both abiotic and biotic factors, especially in dry environments (Wright *et al.* 2002), but at the same time elicits the dilution of metabolic components (high C : N ratio; Garnier & Laurent 1994; Reich *et al.* 1997, 1999; Aerts & Chapin 2000; Mediavilla *et al.* 2001). It therefore seems physically impossible to construct a leaf with long longevity and a high net photosynthetic capacity (Reich *et al.* 1997, 1999). On the other hand, long leaf longevity

increases the retention time of nutrients and has been considered one of the main traits related to long MRT_N (Aerts 1990, 1995; Escudero *et al.* 1992; Eckstein *et al.* 1999). Additionally, low SLA increases resistance to gas exchange, but reduces water loss (Chapin *et al.* 1993; Reich 1998; Reich *et al.* 1997, 1999; Mediavilla *et al.* 2001). Under drought stress the lower water requirements of *Q. ilex* combine with smaller xylem vessels (Villar *et al.* 1997), reducing the risk of embolism (Martínez-Vilalta *et al.* 2002; Tyree 2003). Finally, low SLA and low N concentrations reduce leaf palatability, and this encourages herbivores to feed on more attractive species (Coley 1988).

FINE-ROOT TRADE-OFFS

Quercus faginea had a greater fine-root biomass in spring, lower fine-root tissue density and a higher SRL, which should allow this species to explore larger volumes of soil than *Q. ilex*. The fine-root N content per unit length (mg N m^{-1}) was similar in both species at the time of peak biomass, which suggests that the higher N uptake of *Q. faginea* would have resulted from larger volumes of soil explored by the roots. Owing to the low soil fertility and the strong effect of the water soil content on ion diffusion (Chapin 1991), an extensive fine-root system is probably more effective for absorbing N than a high uptake capacity (high V_{max}) (Aerts & Chapin 2000).

The biomass of fine roots fell drastically with soil water contents in both species throughout late spring and summer, as has been found in seasonal environments (Lamont 1995; Eissenstat & Yanai 1997; López *et al.* 2001). The more pronounced loss of biomass in *Q. faginea* during the dry summer of 1998 points to a greater sensitivity to the soil water content than that seen in *Q. ilex*. During the summer of 1999, biomass loss was similar for both species, but the soil water content was significantly higher than in 1998 (Silla 2001). Lower tissue density has been linked to greater fine-root mortality (Schlöpfer & Ryser 1996), in agreement with our own results. In contrast, working with *Quercus laevis* and *Quercus marilandica*, Espeleta & Donovan (2002) failed to find any relationship between fine-root turnover and tissue density, although the more productive *Q. marilandica* displayed a higher fine-root turnover.

A higher allocation of N and biomass to the fine roots in *Q. faginea* was of substantial importance for taking up greater amounts of N from the soil, thus counteracting greater N loss and maintaining a mean N balance similar to that found in *Q. ilex*. However, in view of the zero nutrient resorption from senescing fine roots reported in different studies (Nambiar 1987; Aerts 1990; Gordon & Jackson 2000), higher N concentrations in the roots of *Q. faginea* inevitably contributed to greater N loss through fine-root litter. Additionally, a lower fine-root density and a longer SRL were linked to greater fine-root biomass loss under severe water stress. If this pattern is indeed general

across a whole range of fast- and slow-growing species, a trade-off between N uptake and the rate of loss should emerge at fine-root level, and would account for part of the whole-plant NUE_N intrinsic trade-off.

TRADE-OFF BETWEEN A_N AND MRT_N AT WHOLE-PLANT AND POPULATION LEVELS

We found an inverse relationship between the components of NUE_N at plant and population levels: *Q. faginea* showed a higher A_N and a lower MRT_N than *Q. ilex*, as predicted (Berendse & Aerts 1987). At population level, when differential plant mortality was included, this trade-off was even more obvious. Few authors have tested this proposal, and the results are inconsistent (Garnier & Aronson 1998; Aerts & Chapin 2000). In work addressing species with contrasting leaf life spans, usually evergreens vs deciduous species (Aerts 1990; Eckstein & Karlsson 1997, 2001; this study), clear trade-offs have been found. When different species with short leaf longevity ranges (<1 year) were compared, this trade-off was seen to be very weak or absent (Aerts & de Caluwe 1994; Vázquez de Aldana & Berendse 1997; Nakamura *et al.* 2002; but see Yasumura *et al.* 2002). As the range of leaf life span broadens, the overall interdependence of leaf life span and other plant traits would provide an increasingly powerful inverse relationship between the components of NUE_N . On the other hand, as the range of leaf life span narrows other sources of variation (e.g. resorption efficiency) become increasingly important in determining leaf and whole-plant trade-offs, and nutrient conservation improves owing to an increasing life span, with no important changes in leaf structure and productivity (Eckstein *et al.* 1999; Eckstein & Karlsson 2001).

Additionally, our results show that the long MRT_N in *Q. ilex* was the result not only of long intrinsic leaf life span, but also of reduced nutrient losses due to harsh environmental factors and herbivores. The dry summer of 1998 reduced the leaf area of *Q. faginea* dramatically and led to the loss of leaves with a high N concentration (Figs 3 and 5); in the worst cases it even caused the death of the plant. Xylem embolism is relatively common under strong drought stress in Mediterranean ecosystems (Lo Gullo & Salleo 1993; Martínez-Vilalta *et al.* 2002), blocking the normal process of leaf resorption (Escarré *et al.* 1999). Nitrogen loss due to caterpillar defoliation was three to four times higher in *Q. faginea* than in *Q. ilex* (Table 4). Herbivory by insects and mammals removes a significant proportion of leaf area in temperate and tropical ecosystems (Lowman & Heatwole 1992; Coley & Barone 1996; Crawley 1997). Early leaf development may cause increased mortality owing to late frosts. Although late frosts did not occur during the years we studied, they are frequent in cold-Mediterranean climates. Late frosts during April 1998 were recorded in a nearby *Quercus pyrenaica* reforested site, causing the death of the first flush of new leaves, which was replaced by a new one

(Silla 2001). Wind damage resulted in the loss of leaf litter with high N concentrations in *Fagus crenata*, and shortened the MRT_N with respect to other species (Yasumura *et al.* 2002). These factors are not usually addressed in most NUE_N experiments, and this may lead to overestimations of MRT_N in productive species, normally characterized by more palatable and fragile leaves. Considering that the absence of a trade-off between the NUE_N components among species with similar leaf spans would be partly due to a high noise/response ratio (Eckstein *et al.* 1999; Eckstein & Karlsson 2001), a better analysis of how biotic and abiotic factors affect NUE_N components should be envisaged in future research.

In our species, the MRT_N had stronger effects than A_N on the NUE_N , as found in some studies (Aerts & de Caluwe 1994; Nakamura *et al.* 2002), but not in others (Aerts 1990; Eckstein & Karlsson 1997; Vázquez de Aldana & Berendse 1997). Here, this resulted in a twofold higher NUE_N in *Q. ilex* than in *Q. faginea*, which emphasizes the importance of long MRT at nutrient-poor sites.

Our work shows that the trade-off posited by Berendse & Aerts (1987) is the result of ecophysiological trade-offs at leaf and fine-root level. In *Q. ilex* the suite of leaf and fine-root traits that allow a long MRT_N and a high NUE_N are consistent with the stress-resistance syndrome proposed by Chapin *et al.* (1993). This suite of traits enables *Q. ilex* to become successfully established in nutrient-poor habitats, with a higher survival than in *Q. faginea*, and to achieve the same biomass and N balance, with less dependence on N uptake. By contrast, *Q. faginea* was more productive but less conservative strategy, showing a stronger dependence on soil resources to compensate for greater N loss.

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