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

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).
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1999; Aerts & Chapin 2000; Wright, Westoby & Reich
2002). This evidence suggests that there should be a
trade-off between plant traits that increase productiv-
ity 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

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 marces-
cent 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 (Garcia-

Our study site was a reforested old field located
12 km south of the city of Salamanca (40°55′ N, 54°0′ 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
crushed 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 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 sub-
samples 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

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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⁻²).

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% ± 0.6 in May to 6.3% ± 0.8 in July; data represent mean water contents at 30 cm depth ± 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:

\[
\text{Leaf expansion rate} = \frac{1}{t_f - t_0} \times 100\ \text{eqn 1}
\]

where \( t_f \) 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:

\[
A_{N(t)} = (B_2 - B_1 + L)\left[rac{(N_{pool2} - N_{pool1})/2}{(N_{pool1}/2)}\right] \text{eqn 2}
\]

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 harvests 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 MRTs is more difficult to calculate properly, even under steady-state conditions (Eckstein et al. 1999; Eckstein & Karlsson 2001). Here the MRTN 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 of 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 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) was calculated as the product of N productivity ($A_N$) and mean residence time (MRT) 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; $P < 0.005$). 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; $P < 0.001$). 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.
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*.

### 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

<table>
<thead>
<tr>
<th>Plant trait</th>
<th>Year</th>
<th>Qi</th>
<th>Qf</th>
</tr>
</thead>
<tbody>
<tr>
<td>Leaf mass area (g g$^{-1}$)</td>
<td>1998</td>
<td>0·317 ± 0·01 (16)</td>
<td>0·172 ± 0·01 (17)</td>
</tr>
<tr>
<td></td>
<td>1999</td>
<td>0·318 ± 0·02 (16)</td>
<td>0·160 ± 0·01 (16)</td>
</tr>
<tr>
<td>Peak plant leaf area (cm$^2$)</td>
<td>1998</td>
<td>227·0 ± 16·3 (21)</td>
<td>320·7 ± 25·0 (28)</td>
</tr>
<tr>
<td></td>
<td>1999</td>
<td>362·8 ± 37·7 (16)</td>
<td>457·3 ± 54·1 (16)</td>
</tr>
<tr>
<td>Peak fine root biomass (g)</td>
<td>1998</td>
<td>1·51 ± 0·10 (38)</td>
<td>1·77 ± 0·11 (39)</td>
</tr>
<tr>
<td></td>
<td>1999</td>
<td>1·73 ± 0·15 (21)</td>
<td>2·24 ± 0·18 (21)</td>
</tr>
<tr>
<td>Specific leaf area (cm$^2$ g$^{-1}$)</td>
<td>1998</td>
<td>56·7 ± 0·99 (25)</td>
<td>91·4 ± 2·04 (16)</td>
</tr>
<tr>
<td></td>
<td>1999</td>
<td>53·6 ± 1·16 (19)</td>
<td>94·9 ± 2·23 (24)</td>
</tr>
<tr>
<td>Daily leaf expansion rate (%)</td>
<td>1998</td>
<td>1·72</td>
<td>2·27</td>
</tr>
<tr>
<td></td>
<td>1999</td>
<td>1·43</td>
<td>2·50</td>
</tr>
<tr>
<td>Leaf N (mg g$^{-1}$) of the new cohort</td>
<td>1998</td>
<td>10·4 ± 0·19 (13)</td>
<td>12·2 ± 0·66 (11)</td>
</tr>
<tr>
<td></td>
<td>1999</td>
<td>9·8 ± 0·40 (21)</td>
<td>12·3 ± 0·40 (20)</td>
</tr>
<tr>
<td>Leaf N (mg cm$^{-2}$) of the new cohort</td>
<td>1998</td>
<td>0·170 ± 0·006 (13)</td>
<td>0·150 ± 0·006 (11)</td>
</tr>
<tr>
<td></td>
<td>1999</td>
<td>0·163 ± 0·006 (21)</td>
<td>0·139 ± 0·007 (20)</td>
</tr>
<tr>
<td>Specific root length (m g$^{-1}$)</td>
<td>1998</td>
<td>2·45 ± 0·17 (35)</td>
<td>3·06 ± 0·26 (34)</td>
</tr>
<tr>
<td></td>
<td>1999</td>
<td>2·32 ± 0·12 (17)</td>
<td>3·10 ± 0·20 (16)</td>
</tr>
<tr>
<td>Fine root tissue density (g cm$^{-3}$)</td>
<td>1998</td>
<td>0·217 ± 0·03 (35)</td>
<td>0·194 ± 0·04 (34)</td>
</tr>
<tr>
<td></td>
<td>1999</td>
<td>0·200 ± 0·02 (17)</td>
<td>0·186 ± 0·02 (16)</td>
</tr>
<tr>
<td>Fine root N content at peak biomass (mg g$^{-1}$)</td>
<td>1998</td>
<td>6·13 ± 0·25 (17)</td>
<td>8·56 ± 0·39 (19)</td>
</tr>
<tr>
<td></td>
<td>1999</td>
<td>4·29 ± 0·22 (19)</td>
<td>5·53 ± 0·21 (20)</td>
</tr>
<tr>
<td>N content per fine root length (mg m$^{-1}$)</td>
<td>1998</td>
<td>2·50</td>
<td>2·79</td>
</tr>
<tr>
<td></td>
<td>1999</td>
<td>1·85</td>
<td>1·78</td>
</tr>
</tbody>
</table>

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).
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) 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 ± 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

<table>
<thead>
<tr>
<th>Parameter</th>
<th>1998</th>
<th>1999</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Qi</td>
<td>Qf</td>
</tr>
<tr>
<td></td>
<td>Qi</td>
<td>Qf</td>
</tr>
<tr>
<td>Plant productivity (g year⁻¹ plant⁻¹)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>New leaf biomass</td>
<td>3·79 ± 0·35</td>
<td>3·41 ± 0·41</td>
</tr>
<tr>
<td>Primary stem growth</td>
<td>0·58 ± 0·06</td>
<td>0·81 ± 0·08</td>
</tr>
<tr>
<td>Secondary stem growth</td>
<td>0·76</td>
<td>0·92</td>
</tr>
<tr>
<td>Lignotuber + secondary coarse root growth</td>
<td>3·77</td>
<td>5·08</td>
</tr>
<tr>
<td>Fine root increment</td>
<td>1·19</td>
<td>1·38</td>
</tr>
<tr>
<td>Plant annual net primary productivity</td>
<td>10·09</td>
<td>11·81</td>
</tr>
<tr>
<td>Population productivity (kg year⁻¹ ha⁻¹)</td>
<td>11·01</td>
<td>11·19</td>
</tr>
<tr>
<td>Population annual net primary productivity</td>
<td>11·22</td>
<td>13·43</td>
</tr>
</tbody>
</table>
Nitrogen losses due to plant mortality were almost three times higher in Quercus faginea than in Quercus 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 POOL 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 Quercus faginea, suggesting a higher N uptake in Quercus faginea than in Quercus 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 Quercus 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 Quercus faginea.

Total N pools were higher in Quercus ilex at population level for both years (Quercus ilex, 78·8 and 115·2 g ha⁻¹; Quercus faginea, 64·2 and 85·9 g ha⁻¹, for 1998 and 1999, respectively), mainly due to the greater mortality of Quercus faginea (Fig. 2) and the subsequent lower density.

### NITROGEN-USE EFFICIENCY

In Quercus faginea, N productivity (A_N) was 24 and 57% higher than in Quercus 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 Quercus 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 a significantly lower N allocation to the canopy in Quercus faginea (Fig. 1). In Quercus ilex, A_N and LNP dropped to around 31–32% in 1999 with respect to 1998, but only to around 12% in Quercus 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, Quercus ilex had an approximately threefold longer mean residence time (MRT_N) than Quercus 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 MRTN in 1998 than for 1999 (Table 4) owing to differences in plant densities.

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Table 3. Means ± 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

<table>
<thead>
<tr>
<th>Level</th>
<th>Parameter</th>
<th>1998</th>
<th>1999</th>
</tr>
</thead>
<tbody>
<tr>
<td>Leaf litter</td>
<td>Biomass losses (g)</td>
<td>0.44 ± 0.06</td>
<td>1.00 ± 0.14</td>
</tr>
<tr>
<td></td>
<td>N losses (mg)</td>
<td>3.09 ± 0.39</td>
<td>7.81 ± 1.43</td>
</tr>
<tr>
<td>Herbivory</td>
<td>Area lost (cm²)</td>
<td>9.3 ± 1.88</td>
<td>35.0 ± 6.14</td>
</tr>
<tr>
<td></td>
<td>N leaf (mg cm⁻²)</td>
<td>20.9 ± 0.9</td>
<td>20.6 ± 0.8</td>
</tr>
<tr>
<td></td>
<td>N losses (mg)</td>
<td>1.94 ± 0.39</td>
<td>7.23 ± 1.27</td>
</tr>
<tr>
<td>Fine roots</td>
<td>Biomass losses (g)</td>
<td>0.50</td>
<td>1.26</td>
</tr>
<tr>
<td></td>
<td>Fine root N (mg g⁻¹)</td>
<td>4.36 ± 0.27</td>
<td>5.26 ± 0.29</td>
</tr>
<tr>
<td></td>
<td>N losses (mg)</td>
<td>2.16</td>
<td>6.64</td>
</tr>
<tr>
<td></td>
<td>Total N losses per plant (mg)</td>
<td>7.19</td>
<td>21.68</td>
</tr>
<tr>
<td>Plant mortality</td>
<td>Mortality (plants ha⁻¹)</td>
<td>95.8</td>
<td>268.3</td>
</tr>
<tr>
<td></td>
<td>Plant N pool (g)</td>
<td>0.072</td>
<td>0.069</td>
</tr>
<tr>
<td></td>
<td>N losses (g ha⁻¹)</td>
<td>6.92</td>
<td>18.63</td>
</tr>
<tr>
<td></td>
<td>Total population N losses (g ha⁻¹)</td>
<td>14.36</td>
<td>35.67</td>
</tr>
</tbody>
</table>

*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.

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

<table>
<thead>
<tr>
<th>Level</th>
<th>Year</th>
<th>Qi</th>
<th>Qf</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plant</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A_N (g g⁻¹ N year⁻¹)</td>
<td>1998</td>
<td>146·1</td>
<td>180.5</td>
</tr>
<tr>
<td></td>
<td>1999</td>
<td>100·9</td>
<td>158·4</td>
</tr>
<tr>
<td>LNP (g g⁻¹ N year⁻¹)</td>
<td>1998</td>
<td>284·4</td>
<td>406·4</td>
</tr>
<tr>
<td></td>
<td>1999</td>
<td>192·2</td>
<td>356·6</td>
</tr>
<tr>
<td>MRT_N (years)</td>
<td>1998</td>
<td>10·05</td>
<td>3·20</td>
</tr>
<tr>
<td></td>
<td>1999</td>
<td>14·11</td>
<td>4·58</td>
</tr>
<tr>
<td>NUE_N (g g⁻¹ N)</td>
<td>1998</td>
<td>1468·6</td>
<td>578·2</td>
</tr>
<tr>
<td></td>
<td>1999</td>
<td>1422·7</td>
<td>725·0</td>
</tr>
<tr>
<td>Population</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A_N (g g⁻¹ N year⁻¹)</td>
<td>1998</td>
<td>146·3</td>
<td>179·3</td>
</tr>
<tr>
<td></td>
<td>1999</td>
<td>100·9</td>
<td>158·4</td>
</tr>
<tr>
<td>MRT_N (years)</td>
<td>1998</td>
<td>5·24</td>
<td>1·54</td>
</tr>
<tr>
<td></td>
<td>1999</td>
<td>14·11</td>
<td>4·58</td>
</tr>
<tr>
<td>NUE_N (g g⁻¹ N)</td>
<td>1998</td>
<td>766·0</td>
<td>277·5</td>
</tr>
<tr>
<td></td>
<td>1999</td>
<td>1422·7</td>
<td>725·0</td>
</tr>
</tbody>
</table>
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 CO2 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 & Heilmeier 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: budbreak 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-Vilalta *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 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 $V_{\text{max}}$) (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 (Schläpfer & 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$_{S}$ 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 $A_{S}$ 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 (Escaré 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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References


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