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Fast growth involves greater use of stored resources for seedling spring shoot growth in Mediterranean evergreen trees

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Abstract

•*Background and Aims* The carbon (C) and nitrogen (N) needed for plant growth can either come from soil N and current photosynthesis or through remobilization of stored resources. The contribution of remobilization to new organ growth on a whole plant basis is quite well known in deciduous woody

plants and evergreen conifers but this information is very limited in broadleaf evergreen trees. We compared the contribution of remobilized C and N to the construction of new organs in spring, and the importance of different organs as C and N sources in one-year-old potted seedlings of four ecologically distinct evergreen Mediterranean trees: *Quercus ilex, Q. coccifera*, *Olea europaea* and *Pinus hapelensis*.

•*Methods* We used a dual ^{13}C and ^{15}N isotope labeling, to disentangle the contribution of currently taken up and stored C and N to new growth. Stored C was labeled under simulated winter conditions.

•*Key results* Oaks allocated most C assimilated under simulated winter conditions in coarse roots while *O. europaea* and *P. halepensis* allocated it in the leaves. Remobilization was the main N source (> 74%) for new fine root growth in early spring but by mid spring, soil supplied most of N required for new growth (> 64%). Current photosynthesis supplied > 60% of the C in new fine roots by mid spring in most species. Across species, the proportion of remobilized C and N in new shoots increased with relative growth rate. *Quercus* species, the slowest growing trees, primarily used currently acquired resources while *P. halepensis*, the fastest growing species, mainly used reserves. Increase in the amount of stored N increased N remobilization, which fostered absolute growth both within and across species. Old leaves were major sources of remobilized C and N but stems and roots also supplied considerable amounts of both compounds in all species except in *P. halepensis*, which mainly relied on foliage formed in the previous growing season to supply stored resources.

• *Conclusions* Seedlings of Mediterranean evergreen trees have distinct C and N storage physiology with growth speed driving the contribution of remobilized resources to new growth.

Key words: ^{13}C; labeling; ^{15}N; *Olea europaea; Pinus halepensis; Quercus ilex; Quercus coccifera;* remobilization; reserves.

1. Introduction

Growth and reproduction in trees consume considerable amounts of carbon (C) and nitrogen (N), which are derived from photosynthesis and soil N absorption and/or the remobilization of internal reserves (Chapin III et al. 1990; Nambiar and Fife 1991; Körner 2003; Millard and Grelet 2010; Brüggemann et al. 2011). Although C and N metabolism are interrelated, storage physiology of both nutrients has important differences. For instance, woody plants store nonstructural C in all organs and remobilization of C reserves is controlled by sink strength. In contrast, storage of N tends to be concentrated in specific organs and N remobilization mainly is source-driven, *i.e.* it depends upon the amount of stored N (Millett et al. 2005; Millard and Grelet 2010).

Storage of C and N is related to leaf habit. Evergreens allocate higher amounts of mobile C to the leaves than do deciduous plants (Chapin III et al. 1990; Cerasoli et al. 2004; Palacio, Millard, et al. 2007), and can assimilate C in winter that contributes to C stores (Hansen et al. 1996; Cerasoli et al. 2004; Kuptz et al. 2011). Conversely, deciduous species tend to store N in woody organs (Millard and Proe 1991; Millard 1996; Silla and Escudero 2003) while leaves, especially the youngest ones, are major N storage sites in evergreen species (Nambiar and Fife 1991; Silla and Escudero 2003). Resprouting ability also affects C and N storage patterns: resprouters generally allocate more C and N to the roots than do nonsprouting species (Palacio, Maestro, et al. 2007).

Studies on the contribution of remobilization to new growth at a whole plant scale have mostly focused on deciduous broadleaf woody species and evergreen conifers (see review by Millard and Grelet 2010 and Brüggemann *et al*. 2011). Little information exists for evergreen broadleaf species (see Cerasoli et al., 2004; Grelet et al., 2001; Silla and Escudero, 2003), even though they are major components of several forests ecosystems worldwide, such as the Mediterranean and tropical biome (Archibold 1995). Overall, remobilization of stored resources is often coupled to plant phenology (Hoch et al. 2003; Körner 2003; Milla et al. 2005) and it also depends upon plant age, life form, growth pattern,

74 climatic conditions, competition and depredation (Chapin III et al. 1990; Maillard et al. 2001; Salifu
75 and Timmer 2003; Millett et al. 2005; Palacio et al. 2014). Plants with a continuous shoot elongation
76 pattern may adjust their resource demand to the acquisition of external resources. In contrast, plants
77 with an episodic shoot growth pattern would depend more on reserves as resource demand is strongly
78 concentrated in a short period (Canham et al. 1999; Salifu et al. 2009). The remobilization of C reserves
79 seems to be more dependent on foliar habit than N remobilization. Specifically, deciduous woody plants
80 seem to greatly rely on C remobilization for early shoot and root growth (Dickson et al. 1990; Sloan
81 and Jacobs 2008; Vizoso et al. 2008; Keel and Schädel 2010). For conifers, however, current
82 photosynthesis seems to be the main C source that fuels early root and shoot growth and stored C
83 becomes more important when current photosynthesis is suppressed (Philipson 1988; van den Driessche
84 1991; Atzmon et al. 1994; Hansen et al. 1996; Brüggemann et al. 2011). However, differences in C
85 remobilization between evergreen and deciduous species may be smaller than initially expected and
86 overridden by inter-species variability (Keel and Schädel 2010; Brüggemann et al. 2011). As
87 comparative studies on C and N remobilization carried out under similar growth conditions and at the
88 whole-plant scale are scarce (Millard et al. 2001; Grelet et al. 2001; Silla and Escudero 2003; Palacio
89 et al. 2014) this makes difficult to draw general ecological patterns on storage physiology.

90 Several studies have addressed the remobilization of stored resources in Mediterranean woody
91 plants (Cherbuy et al. 2001; Silla and Escudero 2003; Milla et al. 2005). However, most of these studies
92 have analyzed remobilization at the branch or leaf level or did not use labeled C or N. This prevents
93 knowing the relative importance of the different plant organs as sources of remobilized C and N and
94 disentangling the contribution of remobilized and current N and C uptake to new growth (Millard 1996).

95 We compared the importance of C and N remobilization for spring growth in the seedlings of
96 four evergreen woody species that coexist in Mediterranean forests and exhibit important functional
97 and ecological differences: *Quercus ilex* subsp. *ballota* (Desf.) Samp. (holm oak), *Quercus coccifera*

L. (kermes oak), *Olea europaea* L. (olive) and *Pinus hapelensis* Mill. (Aleppo pine). Specifically, we investigated whether these species differed in: (1) the allocation pattern of winter-assimilated C; (2) the relative contribution to early growth of new roots and shoots of stored C and N *vs.* current photosynthesis and N uptake; and (3) the importance of different organs as sources of C and N. Three species are shade tolerant broadleaves with resprouting capacity: *Q. ilex* is a late-successional tree that dominates many forest communities in the Mediterranean basin; *Q. coccifera* is a slow growing shrub or small tree common in holm oak and Aleppo pine forests and is a main component of mature plant communities on semiarid sites; *O. europaea* is a mid-successional species and is quite common in holm and cork oak forests on mild winter sites. Finally, *P. halepensis* is a fast-growing, shade-intolerant pioneer non-resprouting conifer, and is very common in disturbed and shallow soils on dry sites in the Mediterranean basin (Blanco et al. 1998). *Quercus* species have strong tap roots that store large amounts of resources and their shoot growth pattern is episodic, while *O. europaea* and *P. halepensis* lack strong tap roots and show a polycyclic shoot elongation pattern (Sánchez-Gómez *et al*. 2006; Willaume and Pagès 2006; Girard *et al*. 2010). We hypothesized that *Quercus* species would be highly dependent on remobilized resources to support new growth in spring, and would preferentially use C and N stored in the roots. We assumed that *P. halepensis* and *O. europaea* would rely more on currently acquired resources. To test these hypotheses, we performed an experiment where seedling C reserves and soil N were labeled with ^{13}C and ^{15}N, respectively. This allowed us to identify and follow through development stage the contribution to the growth of new organs of remobilized C and N, and of current photosynthesis and N uptake.

2. Material and methods

2.1. Plant material

We used one-year-old seedlings, as this development stage is the most critical for plant population dynamics (Pulido and Díaz 2005). Plants were grown for one year in the nursery of the Centro Nacional de Recursos Genéticos Forestales "El Serranillo" in Guadalajara (Spain). Seeding was done in early spring 2003 and the first year cultivation ended in late fall 2003. All plants were cultivated in ®Forest Pot containers (Nuevos Sistemas de Cultivo S.L., Girona, Spain) that has 50 cavities of 300 ml, which were filled with unfertilized peat moss (Kekkilä B0, Kekkilä Oyi, Finland). Seedlings were fertilized weekly for five months from early May 2003 with a 20:3:16, N-P-K water-soluble fertilizer and irrigated every 2–4 days. Most of the cultivation was carried outdoors under full sun conditions but to avoid spring frosts, seedlings were cultivated in a greenhouse until mid May 2003. When seedlings were 11 months old (early January 2004), 60 plants per species were moved to the INRA Institute (Champenoux, France) and placed for 1 month in a controlled environmental chamber (Dagard, Chambres froides modulables, 23600 Boussac, France) for winter acclimation. Day and night chamber temperatures were 8 and 3 °C, respectively, similar to temperatures at many inland Iberian Peninsula locations during winter. The photoperiod was 8h, relative humidity 50–95% and the photosynthetic photon flux density (PPF) was 250 μmol m^{-2} s^{-1}. Seedlings were kept well watered and were not fertilized during this stage. As contribution of the C and N reserves for the new growth was assessed in the second year, we confidently consider that results were not affected by seed carry over effects (Lehmeier et al. 2005; Villar-Salvador et al. 2010).

2.2. Labeling procedure

Fifty-six seedlings per species, homogeneous in size, were chosen for the experiment. Carbon reserves were ^{13}C-labeled by subjecting the seedlings to an enriched $^{13}CO_2$ atmosphere under simulated winter

142 temperature conditions. We ^{13}C-labeled seedlings under simulated winter conditions because we aimed
143 to assess if winter is a high C storage period in evergreen woody species growing under typical
144 Mediterranean continental winter conditions, and because labeling under typical spring conditions has
145 more logistic limitations than performing it under simulated winter conditions. As seedlings showed no
146 growth symptoms both in shoots and roots under simulated winter conditions, we assumed that
147 assimilated C was mostly stored as mobile C rather than transformed into structural carbohydrates.
148 Thirty-six seedlings per species were submitted to four ^{13}C labeling cycles from 9th February to 11th
149 March 2004 in a controlled environment chamber (VTPH 5/1 000, Vötsch Industrie-technik GmbH,
150 Reiskirchen-Lindenstruth, Germany) operating as a semi-closed system (for a full description see Vivin
151 *et al*. 1995), and exposed to $^{13}CO_2$-enriched air (4.4 atom% ^{13}C) at a constant CO_2 concentration of 500
152 ppm. Each labeling cycle took 4 days, and each species was labeled for
153 24 h. The roots were kept isolated from the shoots to prevent any dilution of the enriched 13CO2
154 atmosphere through root respiration. Chamber temperature, relative humidity and PPF were 8±1 °C,
155 77% and 500 μmol m−2 s−1, respectively. Between labeling cycles, the saplings were returned to the
156 growth chamber, under the simulated winter conditions described above.

157 The remaining unselected seedlings were not labeled and were kept under the same simulated winter
158 conditions in a different growth chamber.

159 On March 15 2004, seedlings were transplanted into 3L pots (one seedling per pot) filled with
160 perlite and transferred to a ventilated transparent greenhouse (INRA, Champenoux, France) to promote
161 growth. Plants were arranged in four blocks of eight labeled and three unlabeled seedlings per species.
162 We supplied seedlings with a high fertilization regime to maximize growth. Conversely, soil fertility
163 has little influence on N remobilization (Grelet et al. 2003; Villar-Salvador et al. 2010). From March
164 24 to the end of the experiment on May 2004, each seedling was fertilized daily with 40-80 ml of a
165 complete nutrient solution (Le Blevennec 1986). The nutrient solution contained (mg l^{-1}): KNO_3 (101);

166 $Ca(NO)_2$ (345); K_2HPO_4 (75); H_2KPO_4 (96); $MgSO_4$ $7H_20$ (270); NaCl (10), Mn (0.2), Zn (0.10), Cu
167 (0.012), B (0.10), Mo (0.025) and Fe (2.5). At the end of the experiment, fertilization supplied
168 approximately 12.5 g N m^{-2}. Labeled seedlings were fertilized with the same solution labeled at 2
169 atom% ^{15}N ($K^{15}NO_3$, ^{15}N > 98 atom%, Spectra Stable Isotopes, Division of Spectra Gases Inc.,
170 Columbia, USA).

171 **2.3. Sampling**

172 The plants were harvested at three developmental stages:

173 **t_0:** Before transplanting to the 3L pots and ^{15}N labeling, seedlings showed no growth as they remained
174 under cool conditions.

175 **t_1**: Seedlings had not yet started shoot elongation but had produced significant amounts of new roots,
176 most of which had protruded the plug into the perlite. Most *P. halepensis*, *O. europaea*, *Q. ilex*, and
177 *Q. coccifera* seedlings reached this stage 21, 23, 26 and 31 days after transplanting, respectively.

178 **t_2**: First shoot flush of growth had ceased and most leaves were completely unfolded and mature. Most
179 seedlings in *P. halepensis*, *O. europaea* and *Q. ilex* reached this stage 44 days after transplanting
180 (t_0), while *Q. coccifera* plants reached this stage 59 days after transplanting.

181 Minimum and maximum average temperatures were 11 and 23ºC, respectively at t_1, and 14 and
182 28 °C, respectively at t_2. At each development stage, 10 labeled and four unlabeled seedlings per species
183 were harvested and their roots were carefully washed in tap water to eliminate growing medium, and
184 then rinsed with de-ionized water. Roots were separated into coarse and fine roots. Coarse roots (≥ 2
185 mm in diameter) were only found inside the plugs. Fine roots (< 2 mm in diameter) were classified as
186 protruding fine roots, when they grew out of the plug into the surrounding growing medium and plug
187 fine roots, when they appeared inside the plug. All protruding fine roots were originated during the
188 experiment. Most of the plug fine roots originated during the previous growing season in the nursery
189 but a small fraction of the plug fine roots were formed during the experiment (Figure 1). Shoots were

divided into two parts: stems and leaves that originated in the previous growing season (hereafter stems and old leaves) and stems and leaves that were formed during the experiment, which we considered as a unit (hereafter new shoots). For simplicity, we named the new shoots and new fine roots protruding the plug as new organs, while the rest of the organs were termed old organs because they originated in the previous growing season, although part of their growth occurred during the current growing season. All organs were immediately frozen in liquid N and stored in a freezer at -80 °C, then freeze-dried, weighed and ground in a ball mill (Sodemi, St Ouen L´Aumône, France) for C and N analyses.

2.4. Isotopic analyses and calculations

Total C and N concentrations, and $^{13}C/^{12}C$ and $^{15}N/^{14}N$ isotopic ratios were measured with an elemental analyzer (NA 1500 NCS, Carlo Erba, Milan, Italy) coupled to a Delta-S isotopic ratio mass spectrometer (Finnigan–Mat, Thermoquest Corp., San Jose, CA) at the Plateforme Technique d'Ecologie Fonctionnelle (OC 081, INRA, Champenoux, France).

The contribution of both currently assimilated C under simulated winter conditions (hereafter winter C) to reserves and C from reserves and soil N to seedling growth in spring conditions was calculated with isotopic dilution equations in two different steps (Deléens et al. 1994). It can be expected that a fraction of C and N found in an organ comes from currently assimilated C and N taken up from the fertilizer (*X*), respectively, while the reminder C and N derives from remobilized compounds (*Y*) that were stored before t_0, which in our case are labeled C and unlabeled N, respectively. For either C or N, $X+Y=1$, where (*X)* can be calculated in each organ from:

$$A\%_{organ} = X \times (A\%_{new}) + Y \times (A\%_{old}) \quad (1)$$

$$X = \frac{A\%_{organ} - A\%_{old}}{A\%_{new} - A\%_{old}} \quad (2)$$

where, firstly to calculate the fraction of winter C in an organ at t_0, $A\%_{reserves}$ and $A\%_{new}$ are the ^{13}C abundance of unlabeled plants at t_0 and of the $^{13}CO_2$-enriched air (4.4 atom% ^{13}C)

used in the labeling chamber, respectively. Secondly, to calculate the contribution of C and N reserves for new growth, $A\%_{organ}$ is either ^{13}C (or ^{15}N) abundance in the organ at a specific developmental stage and $A\%_{old}$ is the ^{13}C abundance at t_0 of the ^{13}C-labeled organ (or the ^{15}N abundance of the unlabeled organ). We assumed that $A\%_{old}$ of an organ corresponded to compounds from reserves (C or N) and that the values were equal to A% of the bulk plant material (Cerasoli et al. 2004). For C, $A\%_{new}$ is ^{13}C abundance of the organ in unlabeled seedlings at a specific developmental stage. For N, $A\%_{new}$ is the ^{15}N abundance of the labeled fertilizer.

The new C and N content of each seedling organ were calculated as the product of X (X_C for C and X_N for N) and the C or N content of the organ (organ mass × N or C concentration). The old C and N content of each organ was calculated as the difference between its total C or N content and its new C or N content, respectively.

Plant N uptake rate (N_u) between consecutive developmental stages was calculated as:

$$N_u = \frac{\text{Plant new N content}_{t_{n+1}}}{t_{n+1} - t_n} \times \frac{1}{\text{Fine root mass}_{t_n}} \quad (\text{mg mg}^{-1}\ \text{d}^{-1}) \qquad (3)$$

Relative growth rate (RGR) between t_0 and t_1 or t_2 was calculated as:

$$\text{RGR} = \frac{\ln(\text{Plant mass}_{t_i}) - \ln(\text{Plant mass}_{t_0})}{t_i - t_0} \quad (\text{mg g}^{-1}\ \text{d}^{-1}) \qquad (4)$$

where t_i is either t_1 or t_2. Partitioning of new C ($P_{C\,new}$) and new N ($P_{N\,new}$) into the organs at each sampling date was determined as:

$$P_{new} = \frac{\text{Content of new C or N in an organ}}{\text{Content of new C or N in the plant}} \times 100 \quad (\%) \qquad (5)$$

Plant respiration was estimated as the difference between the reduction in old C

content in old organs (coarse roots, plug fine roots, stems and old leaves) between t_0 and t_2 and the amount of old C in the new organs (new shoots and protruding fine roots) at t_2.

2.5. Statistical analyses

Preliminary analyses showed that the block was not statistically significant in any case so for simplicity it was excluded from subsequent analyses. We used a one-way ANOVA to assess species effect on the content of labeled C recovered after labeling at t_0. The effect of species and development stage on plant organ mass, X_C, X_N, new and old C and N content were assessed by two-way ANOVA for each plant organ separately.

To assess if a given species favored specific plant organs for winter C storage, we quantified both the absolute (observed winter C) and the relative amount of winter C in an organ after ^{13}C labeling. The null hypothesis was that organ sink strength for winter C is directly proportional to its mass. If an organ is a priority site for storage it would have higher amount of winter C than predicted by its mass. Thus, to quantify the relative amount of winter C we compared the observed winter C content with the predicted winter C content in an organ, assuming that the allocation of currently fixed winter C was proportional to the mass of the organ. Predicted winter C content of an organ *i* after labeling was calculated as:

$$\text{Predicted winter C}_{\text{organ}_i} = \frac{\text{Organ}_i \text{ mass}}{\text{Plant mass}} \times \text{Plant winter C content} \quad \text{(mg)} \quad (6)$$

For winter C content we performed an ANOVA test for each species with plant organ and the interaction between plant organ and observed/predicted factors in the model. Fisher's Least Significant Difference test was used to identify differences between observed and predicted means in each plant organ. For all our analyses, data homocedasticity was checked with the Levene test. When ANOVA assumptions were not met, data were transformed.

Relationships between variables were analyzed with the Pearson correlation or the Kendall Tau correlation when relationships were nonlinear. Statistical analyses were conducted with the STATISTICA 7.0 software (StatSoft, Inc, Tulsa, USA).

3. Results

3.1. Growth [1]

At t_0, *P. halepensis* seedlings were the smallest and *Q. ilex* seedlings were the largest seedlings, while *O. europaea* and *Q. coccifera* seedlings were intermediate in size (Figure 1). Coarse roots in both oaks were larger than in *O. europaea* and *P. halepensis*, which proportionally had more mass in old leaves and stems than *Quercus* species. At t_2, *O. europaea* had the highest and *Q. coccifera* the lowest absolute growth (59±9.9 and 24±3.8 mg for *O. europaea* and *Q. coccifera*, respectively), while absolute growth of *Q. ilex* and *P. halepensis* were 43±7.3 and 39±3.2 mg, respectively ($F_{3,54}$=678; P<0.001). The oaks, especially *Q. coccifera* had lower RGR than the remaining species. At t_2, *P. halepensis* had the highest RGR while RGR in *O. europaea* was intermediate (Table 1).

Overall, the mass of most organs increased through development stage (Figure 1; P<0.001) similarly across species except for *Quercus* species whose coarse root mass increase was the highest (Development stage × Species interaction, $F_{6,119}$=2.83; P<0.01).

The mass of fine roots protruding the plug, was highest in *Q. coccifera* and lowest in *Q. ilex* at both development stages, whilst *O. europaea* and *P. halepensis* had intermediate mass values with very little difference between them ($F_{3,66}$=3.83; P=0.014) (Figure 1). New shoots appeared between t_1 and t_2 and were two to five times larger than the fine roots protruding the plug. New shoots were bigger in *Q. ilex*

[1] Preferred position for Figure 1.

and *O. europaea* than in *Q. coccifera* and *P. halepensis*, whose new shoot growth was similar ($F_{3,33}$=3.84; P=0.012)..

3.2. C allocation under winter conditions[2]

After ^{13}C labeling under simulated winter conditions, the amount of C incorporated (winter C) by the seedlings varied among species in the following order *P. halepensis* > *Q. ilex* > *O. europaea* > *Q. coccifera* (Figure 2). In both *Quercus* species most winter C was allocated to coarse roots, the largest plant organ. However, in *Q. ilex*, the old leaves contained more winter C than predicted by their mass, while the fine roots confined in the plug contained less winter C than predicted. In contrast, winter C was allocated proportionally to the size of each organ in *Q. coccifera*. Unlike the oaks, most winter C in *O. europaea* and *P. halepensis* was allocated into old leaves, which also contained more winter C than predicted by their mass. On the other hand, roots in *P. halepensis* and stems and plug fine roots in *O. europaea* contained less winter C than predicted.

3.3. New and old C composition of plants[3]

Overall, old leaves, new shoots and protruding fine roots had the highest X_C values, while the fine roots inside the plug had the lowest X_C values. In most organs, the fraction of new C (X_C; C derived from current photosynthesis) increased throughout development, except in *Q. ilex* where no change in X_C was noted (Table 1).

The contribution of new C to new shoot and protruding fine roots growth was generally greater than the contribution of old C. At t_1, new C represented most (> 50%) of the C content in *Q. ilex* protruding fine roots, it was less than 50% in *P. halepensis* and *Q. coccifera* while in *O. europaea* new C represented less than 20% (Table 1) (see also Figure S2 in Supplementary Material). At t_2, X_C in fine roots that protruded the plug was greater than 60% in all species except for *Q. coccifera*, where it

[2] Preferred position for Figure 2.

[3] Preferred position table 1and figure 3

represented less than 50%. In all species, new C in new shoots represented around 50% or more of total C except for *P. halepensis*, where it was < 40%. Globally, *Q. coccifera* had the highest X_C and *P. halepensis* the lowest, while *Q. ilex* and *O. europaea* had similar X_C values, intermediate between *Q. coccifera* and *P. halepensis*.

Although total C (new + old C) in the plant increased throughout development, plant old C content decreased and differed among species (Figure S1 a and c and Table S1 in Supplementary Material). In all species, old leaves experienced the greatest reduction in old C content (Figure 3). Coarse roots in the oaks and *O. europaea* and stems in *Q. ilex* also showed a strong decrease in old C. Part of the decrease in old C was remobilized to supply new growth but part was respired, exuded or volatilized because the amount of old C recovered from new organs at the end of the study was lower than total old C reduction in old plant organs and part. The amount of remobilized C at t_2 was lower in the two *Quercus* species than in *O. europaea* and *P. halepensis*, which were similar (Figure 3; $F_{3,26}$=70.8; P<0.001). The amount of C lost from plant also differed among species at t_2. It was highest in *Q. ilex*, who respired 2.3, 3.7, and 4.1 times more C than *Q. coccifera*, *O. europaea* and *P. halepensis*, respectively ($F_{3,26}$=204; P<0.001).

For all species, most new C was partitioned into woody organs (coarse roots and stems) and fine roots in the plug (Table 1). The highest partitioned new C at t_2 were observed for *P. halepensis* and *Q. ilex*, the lowest values for *O. europaea*, and intermediate values were found for *Q. coccifera*.

3.4. New and old N composition of plants

The fraction of new N (X_N; N taken up from the soil) increased in most organs throughout development with differences among species (Species × Development stage interaction, Table 1). X_N was lower in coarse roots, stems and plug fine roots than in new shoots and protruding fine roots. At t_1, protruding fine roots in *Q. coccifera* had the highest X_N values and *P. halepensis* the lowest values, whilst *O. europaea* and *Q. ilex* had intermediate X_N values. At t_2, *Q. coccifera* and *P. halepensis* had the highest

X_N values (>85%) in protruding fine roots, while *O. europaea*, and especially *Q. ilex*, had the lowest X_N values (Table 1). Most N in new shoots of *Q. coccifera* was new. In contrast, new N represented only a small fraction of the N in new shoots in *P. halepensis*, while the percentage was intermediate in *O. europaea* and *Q. ilex*.

At t_1, > 87% of new N was partitioned into the old organs, i.e. those originated in the previous growing season: coarse roots, stems and the fine roots confined in the plugs. But at t_2, partitioning of new N into old organs strongly decreased. *Pinus halepensis* had the highest and oaks, especially, *Q. coccifera*, the lowest values, while *O. europaea* showed intermediate values of N partitioning into old organs (Table 1).

Plant old N content differed among species as follows: *O. europaea* > *Q. ilex* > *P. halepensis* ≥ *Q. coccifera* but did not change through development (Figure S2 a and c in Supplementary Material). *Olea europaea* had the highest N remobilization from old organs, while *Q. ilex* and *P. halepensis* had intermediate values, and *Q. coccifera* the lowest (Figure 3). Old leaves supplied most remobilized N in all species but this was notorious in *P. halepensis*. Plug fine roots in *Q. coccifera*, *Q. ilex* and *O. europaea* and stems in *Q. ilex* and *O. europaea* were also significant contributors of remobilized N. In both oaks, surprisingly little N was remobilized from coarse roots despite their high N content.

N_u was greater from t_1 to t_2 than from t_0 to t_1, when N_u values were 0.05, 0.14 mg N mg^{-1} d^{-1} for *P. halepensis* and *Q. coccifera*, respectively and 0.17 mg N mg^{-1} d^{-1} for both *Q. ilex* and *O. europaea*. From t_1 to t_2, N_u values were 0.16, 0.31, 0.38 and 0.44 mg N mg^{-1} d^{-1} for *P. halepensis*, *Q. ilex*, *O. europaea* and *Q. coccifera*, respectively.

3.5. Relation between variables[4]

Across species, both X_N and X_C in new shoots (Figure 4a and b) were negatively related to RGR. Partitioning of new N into old organs (coarse roots, plug fine roots, stems and old leaves) was positively related to RGR (Figure 4c) and negatively related to X_N in new shoots (Figure 4d). Conversely, partitioning of new C into old organs was not related either to RGR and X_C in new shoots. Remobilized C and N at t_2, was not related either to RGR or X_C and X_N in new shoots, respectively. Absolute growth was positively correlated with the old N content of the seedlings at both the intra- and inter-specific levels (Kendall Tau=1, P=0.042; Figure

4e). Finally, absolute growth was positively related to remobilized N across species (Figure 4f) but not to remobilized C (data not shown). The amount of new C recovered in the plant relative to the C used for plant growth and reserve replenishment at t_2 was 94, 87, 81 and 79% for *Q. coccifera*, *Q. ilex*, *O. europaea* and *P. halepensis*, respectively. This fraction of new C in the plant was negatively related to RGR (Tau=-1; P=0.042).

4. Discussion

Previous studies have shown important differences in N and C remobilization between deciduous and evergreen conifers (Silla and Escudero 2003; Keel and Schädel 2010; Millard and Grelet 2010; Brüggemann et al. 2011). We report that seedlings of coexisting Mediterranean evergreen woody plants have distinct C and N storage physiology. These differences were found in the way seedlings store winter C, how they use remobilized C and N for new spring growth and the contribution of plant organs to C and N remobilization.

[4] Preferred position for figure 4.

Storage pattern of winter-assimilated carbon

All species assimilated labeled C under simulated winter conditions. Evergreen trees from other temperate biomes are also known to assimilate C during winter as long as low temperatures do not limit photosynthesis (Hansen et al. 1996; Körner 2003). As seedlings showed no growth symptoms under winter conditions, assimilated C probably was mostly stored as mobile compounds rather than transformed into structural carbohydrates. Some of the stored winter C was consumed by respiration or exuded during spring and the remainder was used to fuel spring growth.

Winter C storage pattern across species reflected species differences in seedling morphology. Overall, the amount of winter C stored in the different organs was directly related to their mass. Leaves were the priority sites for winter C storage in *P. halepensis* and *O. europaea* seedlings because they were main C sinks but also because they contained more winter C than the predicted from their mass (Figure 2). Hansen et al. (1996) found contrary results for 3-year-old *Pinus sylvestris* L. saplings; the roots were the main sink for winter C while foliage played a secondary role. This suggests that winter C allocation may differ among *Pinus* species at early development stages. In contrast to *P. halepensis* and *O. europaea*, the coarse roots, the largest organ in these seedlings, were the main C sink in the oaks. *Quercus rubra* L. seedlings also allocate almost all fixed C to roots when shoot elongation is arrested (Dickson *et al*. 1990). Similarly, shade-tolerant broadleaf with episodic shoot growth, such as oaks, also show greater root C storage than do continuously growing, shade-intolerant trees (Canham et al. 1999). Despite their relatedness, both oaks differed in their allocation of winter C. *Quercus ilex* allocated more winter C to foliage than predicted from its mass at the expense of allocation to fine roots, whereas in *Q. coccifera*, the amount of C allocated closely matched organ mass. Our results for *Q. ilex* are consistent with findings for the Mediterranean oak, *Q. suber*, which concentrated 30% of winter C in leaves although foliage only represented 21% of plant mass, while the roots, representing 62% of the plant mass, contained less than 50% of the winter C (Cerasoli et al. 2004).

The relative contribution to growth of new roots and shoots of stored C and N

Growth of new roots and shoots in early spring is crucial for seedling establishment and survival to summer drought in Mediterranean ecosystems (Padilla and Pugnaire 2007; Villar-Salvador et al. 2012). This study shows that remobilization of stored C and N played an important role in this new growth but with notable differences among species, organs and between developmental stages. For instance, soon after transplanting in early spring remobilized N and C were the main source for the growth of new roots (the fine roots protruding the plug into the surrounding soil) in all species seedlings; then by mid spring, most N and C in protruding fine roots derived from the soil or current photosynthesis, as indicated by low X_N and X_C values at t_1 and high at t_2 (Table 1). In general, the contribution of C reserves to new organ construction was greater than the contribution of stored N. Moreover, remobilization of N, but not of C, increased the absolute growth of seedlings at a withinspecies level supporting the idea that absolute growth is a N-source driven process (Millard and Grelet 2010) supporting the idea that C and N have some differences in their storage physiology. Very interesting, we also found evidence that the amount of remobilized N to new organs may also drive seedling absolute growth differences across species (Figure 4).

A major finding of this study is that the composition of seedling new shoots in remobilized C and N increases with species RGR (see Figure 4 and Figures SI and SII in supplementary material). For the fastest-growing species, *P. halepensis*, remobilization was responsible for most C and N in new shoots while protruding fine roots contained mostly recently assimilated C and N. This resulted in very different proportions of new C and N in new shoots and in protruding fine roots for *P. halepensis*. By contrast, both new shoots and protruding fine roots were enriched in new C and N in oaks, the slowest-growing species, indicating that current photosynthesis and soil N were the main sources for the growth of these new organs. Finally, *O. europaea* had intermediate growth and intermediate values for the proportion of new C and N in new shoots and protruding fine roots. Similar to our findings for the oaks,

413 the contribution of remobilized C to new leaf and stem growth was low in spring for *Q. suber*, *Pinus*
414 *nigra* Arn. subsp. *laricio*, and *Pinus uncinata* Ramond (Cerasoli et al. 2004; Maillard et al. 2004; Felten
415 et al. 2007). In the evergreen *Vaccinium vitis-ideae* L.,
416 N remobilization supplied 47-69% of N in new shoots shortly after first flush (Grelet *et al*. 2001), which
417 is similar to our values for *Q. ilex*, *O. europaea* and *P. halepensis*. In *P. nigra* remobilized N made up
418 approximately 70 and 20% of new shoot and new root N, respectively (Maillard et al. 2004). In contrast,
419 a lower proportion of the N consumed for spring leaf growth (32%) in *Q. rubra* was met by N
420 remobilization (Salifu et al. 2008).

421 The higher proportion of remobilized C in new shoots with RGR increase is likely the result of
422 current photosynthesis not meeting C requirements for new organ growth in the faster-growing species,
423 which led to greater support by stored C. This notion is supported by the fact that the new C acquired
424 by the plant was not enough to fulfill the C used for plant growth and storage replenishment, especially
425 in the fastest growth species (*O. europea* and especially *P. halepensis*). Silla and Escudero (2003)
426 concluded that N remobilization in Mediterranean oaks increases when N taken up from the soil does
427 not meet plant N demand. We consider that N uptake *per se* did not limit the growth of new fine roots
428 and shoots in our study as N taken up by roots (total new N in the plant) represented 84-94% of the N
429 content in new organs in early spring, and 98-137% in mid spring across species (data not shown).
430 Thus, why did fast-growing species use less N from the soil for new shoot growth and rely more upon
431 remobilization? We suggest that the underlying mechanism that explains the higher proportion of
432 remobilized N in new shoots with RGR increase is that old organs (old leaves, plug fine roots and
433 woody organs such as coarse roots and stems) are strong N sinks of recently taken up N in fast growth
434 species. This likely reduces the amount of soil N remaining for new organ growth and consequently,
435 increases the dependence on remobilized N to meet new organ N demand. Three results support this
436 hypothesis. Firstly, in all species N remobilization in early spring (t_1) sustained the growth of protruding

fine roots, which was a very weak N sink when compared to old organs that coped with most taken up N as indicated by very high new N partitioning (> 87%) in these organs (Table 1). Consequently, the amount of new N available for allocation to protruding fine roots was very low, contributing only 2136% of these fine root N. Secondly, sink strength of old organs for new N was higher in fast growing species as evidenced by greater partitioning of soil N to these organs with increasing RGR (Figure 4d). Thus, less new N was available for new shoot and protruding fine root growth due to high allocation of new N in old organs in faster growing species. Radial growth in woody organs and replenishment of N reserves likely explain the high N allocated into old organs in spring (Kagawa et al. 2006). Thirdly, the N sink strength of old organs increased with RGR. Specifically, the amount of N demanded by old organs was similar to the N in new organs in *P. halepensis* (the fastest growth species), it was 2.2 and 2.3 times lower in *O. europaea, Q. ilex*, respectively and finally it was three times lower in *Q. coccifera* (the slowest growth species), respectively.

Differences among species in N_u or the timing of organ growth and replenishment of N reserve might also have affected N remobilization by altering N source-sink relations (Nambiar and Fife 1991; Hansen et al. 1996; Dyckmans and Flessa 2001; Willaume and Pagès 2006). For instance, N_u was lowest in *P. halepensis*, the fastest growing species, while oaks with the lowest RGR, had higher N_u; this higher N uptake rate might have alleviated the N remobilization dependence in the oaks. Unfortunately our study has not suitable temporal resolution to assess the overlap of N consumption in different organs.

Our findings have interesting ecological and functional implications. To our knowledge, this is the first time that a relationship has been described between the contribution of remobilized C and N to new organ growth and RGR. We suggest that remobilization could be part of the suite of traits that conform the “fast-growth syndrome” in plants such as leaf specific area or leaf area ratio (Cornelissen et al. 1996; Antúnez et al. 2001). Fast-growing species, such as *P. halepensis*, that rapidly colonize

461 disturbed areas (Barbéro et al. 1998), may rely on remobilized resources to support fast new shoot
462 growth in spring, which can increase performance in competitive environments (Bausenwein et al.
463 2001) and help seedling establishment in spring. By contrast, slow-growing species, such as evergreen
464 oaks, are likely to rely more on current photosynthesis and soil N to support new growth, with
465 remobilization playing a secondary role. Because the concentration of stored C and N (Sala et al. 2012;
466 Gilson et al. 2014) increases, while the proportion of foliage and RGR (Poorter et al. 2012) decreases
467 with tree age, the relationship between new organ composition in remobilized C and N and RGR might
468 also change along plant ontogeny. These ideas should to be tested in a higher number of species and in
469 species from other biomes.

470 Results did not support our initial hypothesis that oaks would rely more on reserves to support
471 new growth in spring. Oaks seedlings probably use a low amount of stored resources for respiration
472 and seasonal cold and drought acclimation, and reserve a high proportion of their pools of stored
473 resources (especially N in coarse roots, Figure 3) for recovery after unpredictable disturbances or to
474 persist under prolonged stress conditions (Canham et al. 1999; del Tredici 2001).

475 Understanding how seedlings use stored resources to support growth is of practical importance
476 for cultivating high quality seedlings. Survival of planted seedlings in dry sites is linked to their capacity
477 to produce new shoots and large and deep root systems before the onset of the dry season (Padilla and
478 Pugnaire 2007; Villar-Salvador et al. 2012). Our results suggest that nursery practices should promote
479 C and N storage in *P. halepensis* prior to planting, because this species relies heavily on stored reserves
480 for new growth. This can be done using high N fertilization levels or specific fertilization regimes
481 (Villar-Salvador et al., 2012). Similarly, nurseries should promote traits conferring high C assimilation
482 and soil N acquisition in oaks, especially for *Q. coccifera*, which primarily use external C and N sources
483 to support new growth. This can be achieved by producing seedlings with high amount of leaves of
484 high carboxylation capacity and promoting large root systems using large volume containers

(Domínguez-Lerena et al. 2006; Villar-Salvador et al. 2012). Finally, both C and N storage and high external acquisition capacity must be promoted in *O. europaea*, which use both sources equally to supply new organ growth.

Importance of different organs as sources of C and N

Part of the C released from old organs (Figure S1) was respired or given off as exudates or volatiles (Loescher et al. 1990) and part was remobilized to fuel new growth. Unfortunately, we were not able to assess the contribution of each organ to total remobilized C as we did not distinguish between respired and remobilized C. In all species, old C stores were to a great extent replenished by currently fixed C, as observed for other species by Chapin III *et al*. (1990), Loescher *et al*. (1990), Cerasoli *et al*. (2004). Though remobilized C did account for most of the reduction in old C on *P. halepensis* and *O. europaea*, C remobilization was lower in the *Quercus* species where most old C was respired during spring growth. Contrary to our results in the *Quercus* species, Cerasoli *et al*. (2004) found that stored C did not fuel respiration during spring growth in the evergreen oak *Q. suber*.

Leaves are usually the main sites for C and N remobilization in evergreen woody plants, with woody stems and roots playing the major role in deciduous species (Nambiar and Fife 1991; Millard and Proe 1993; Millard et al. 2001; Grelet et al. 2001; Palacio, Millard, et al. 2007). In our study, old foliage of seedlings was indeed a major source of C and N: leaves showed the greatest reduction in C and N amounts. Old foliage supplied *ca.* 84 and 61% of remobilized N in *P. halepensis*, *Q. ilex*, respectively. A similar pattern was reported for *P. sylvestris* and *Q. suber* (Millard et al. 2001; Cerasoli et al. 2004). In contrast, old foliage only supplied 56 and 33% of remobilized N in *Q. coccifera* and *O. europaea* highlighting the importance of other plant organs as sources of remobilized N such as coarse roots and stems (Silla and Escudero 2003). Only the mobile fraction of organ C and N can be remobilized. As organs contain different amounts of mobile C and N (Valenzuela 2006), this hinders comparing the relative importance of organs for C and N remobilization across species.

5. Conclusions

We have shown that winter C accumulated throughout the seedling in all species but the amount of allocated winter C mostly depended on the size of the organ. However, leaves are priority winter C storage sites in most species as C is accumulated more than predicted by foliage mass. Most of the C assimilated under simulated winter conditions was presumably respired, exuded or and part of the remainder was used to increase C reserves, being used for new growth in spring. Remobilization was the main N source for the fine roots protruding the plug into the surrounding soil in all species soon after transplanting, but soil N supplied most N in these fine roots in mid spring. The contribution of stored C to new organ construction was generally smaller than the contribution of currently fixed C and this difference was more apparent in mid spring. Absolute growth increased as stored and remobilized N increased both within and across species. However, species RGR determined the contribution of remobilized resources to the construction of new shoots, with fast-growing species using greater proportion of remobilized resources than slow-growing species. Old leaves were important sources of remobilized C and N for all species, but woody organs (coarse roots and stems) also played an important role in most species. These results evidence that the seedlings of Mediterranean evergreen trees differ in their C and N storage physiology, which may reduce competition for soil N in spring facilitating species coexistence.

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684

Figures and tables

Table 1. Fraction of new C (X_C; unlabeled C derived from current photosynthesis) and N (X_N; labeled N taken up from the soil) in different plant organs for *Quercus coccifera*, *Q. ilex*, *Olea europaea* and *Pinus halepensis* seedlings sampled before shoot elongation (t_1) and at the end of the first shoot flush (t_2). Values are means ± 1 standard error. F-values and significance results of two-way ANOVA are shown in the last three columns. Where *: 0.01<P≤0.05; **: 0.001<P≤0.01; ***: P≤0.001, ns: P>0.05. #.Old organs are coarse roots, fine roots in the plug, stems and old leaves, originated in the previous growing season.

	Q. coccifera		*Q. ilex*		*O. europaea*		*P. halepensis*		Species (1)	Develop. stage (2)	1 × 2
	t_1	t_2	t_1	t_2	t_1	t_2	t_1	t_2			
RGR (mg g^{-1} d^{-1})	0.5±0.9	2.8±0.7	2.8±0.8	4.4±0.4	8.6±0.8	7.4±0.5	7.5±1.2	10.6±0.6	32.0***	5.6**	3.2*
X_C (%)											
New shoots		82±4.4		57±7.2		47±3.2		39±4.1	14.9 ***		
Old leaves	52±2.6	66±3.7	51±4.5	49±3.3	27±3.7	40±5.5	24±2.7	37±4.1	25.0 ***	12.4 *	2.27 ns
Stems	9±2.7	34±6.9	45±3.2	34±4.7	6±1.4	7±1.6	23±4.0	33±3.9	31.1 ***	7.7 **	8.4 ***
Coarse roots	10±0.9	20±4.9	21±1.7	23±2.4	12±3.2	24±5.4	29±3.9	41±3.8	12.8 ***	13.6 ***	1.09 ns
Plug fine roots	6±1.1	14±4.8	8±2.0	7±0.8	14±3.6	22±6.7	26±2.9	23±2.6	8.1***	3.91*	0.67 ns
Protruding fine roots	38±4.3	46±6.5	64±3.3	62±5.4	16±3.5	61±2.1	45±4.2	88±0.6	23.6 ***	59 ***	17.8 ***
Partitioning of new C into old organs# (%)	99±0.2	76±4.3	99±0.3	85±1.7	99±0.1	64±3.7	98±0.4	85±1.3	2.6*	794***	15.6***
X_N (%)											
New shoots		70±4.0		66±4.0		52±2.9		22±2.5	27.6 ***		
Old leaves	2±0.3	7±1.1	2±0.2	3±0.5	5±0.5	5±0.7	2±0.2	16±2.0	12.6 ***	103***	22.7 ***
Stem	11±1.1	31±2.4	10±1.2	16±1.2	6±0.8	20±1.2	4±0.4	25±1.6	17.7 ***	272 ***	14.8 ***
Coarse roots	9±1.0	24±2.6	9±1.0	9±1.1	6±0.7	17±1.4	8±0.5	27±0.9	14.4 ***	135 ***	14.6 ***
Plug fine roots	10±0.8	28±2.8	9±0.8	14±1.6	11±1.2	31±2.9	5±0.3	20±0.8	20.9 ***	226 ***	10.6 ***
Protruding fine roots	43±7.3	88±2.8	25±2.6	69±4.4	24±2.4	72±4.9	19±1.7	89±2.2	10.5 ***	292 ***	5.5 ***
Partitioning of new N into old organs# (%)	90±1.9	31±2.6	94±1.2	32±3.8	93±1.2	41±3.2	87±1.1	47±1.5	4.3 **	1098***	10.0***

Figure captions

Figure 1. Dry mass of different plant organs at different growth stages in *Quercus coccifera*, *Q. ilex*, *Olea europaea* and *Pinus halepensis* seedlings. (t_0): after ^{13}C labeling and before spring growth conditions; (t_1): shoot elongation had not yet started but seedlings had produced significant amounts of new fine roots that protruded the plug; (t_2): first shoot flush of growth had finished and most leaves had completely unfolded and matured. Data are means ±1 SE (n=14).

Figure 2. Observed *vs.* predicted (according to organ mass) labeled ^{13}C content in different organs after labeling under simulated winter conditions in seedlings of *Quercus coccifera*, *Q. ilex*, *Olea europaea* and *Pinus halepensis*. In each subfigure, the total content of labeled ^{13}C per plant is shown. Data are means ± 1 SE (n=10). The effects of organ and the organ × observed / predicted factors on labeled ^{13}C content are shown in each subfigure. For each plant organ, an asterisk indicates significant differences between observed and predicted results.

Figure 3. Reduction in old C (calculated as the difference between labeled C content at t_0 and labeled C content at t_2) and remobilized N (calculated as the difference between unlabeled N content at t_0 and unlabeled N content at t_2) content in old organs (coarse roots, plug fine roots, stems and old leaves) in *Quercus coccifera*, *Q. ilex*, *Olea europaea* and *Pinus halepensis* seedlings at the end of the study. Part of C reduction at plant level was due to remobilization and part was lost as respiration, volatiles or exudates. The C amount of both fractions is indicated in each subfigure. Data are means ± 1 SE (n=10).

Figure 4 Relationship between the relative growth rate (RGR) across species and the fraction of new C (X_C; current photosynthesis) in new shoots (a); and fraction of new N (X_N; soil N) in new shoots; (b) and partitioning of N taken up from the soil into old organs (coarse roots, plug fine roots, stems and old leaves) ($P_{new\ N,old\ organs}$) (c). Subfigure

(d) shows the relation between partitioning of N taken up from the soil into old organs

($P_{new\ N,old\ organs}$) and fraction of new N (X_N; soil N) in new shoots. Subfigure (e) presents the relation between plant absolute growth and plant old N content at the within-species scale (inter-specific scale in inserted figure). Finally, subfigure (f) represents the relation between absolute growth and remobilized N across species. Each point in subfigures (a), (b), (c), (e) and (f) is the species mean value and bars are 1 SE. In subfigure (e), each point represents one plant and the Pearson correlation coefficient for each species is: *Q. coccifera* (r=0.89; P=0.02), *Q. ilex* (r=0.81; P=0.03), *O. europaea* (r=0.80; P=0.06) and *P.*

halepensis (r=0.95; P=0.001).

Figure S1. New (unlabeled C, upper figures) and old C (labeled C, bottom figures) content in new organs (new shoots and fine roots protruding the plugs into the surrounding soil) (right figures) and old organs that mostly originated in the previous growing season (coarse roots, plug fine roots, stems and old leaves) (left figures) of *Quercus coccifera*, *Q. ilex, Olea europaea* and *Pinus halepensis* seedlings measured at different growth stages. (t_0): after ^{13}C labeling and before spring growth conditions; (t_1): shoot elongation had not yet started but seedlings had produced significant amounts of fine roots that protruded the plug; (t_2) first shoot flush of growth had finished and most leaves had completely unfolded and matured. Data are means ±1 SE (n=10).

Figure S2. New (labeled N; upper figures) and old (unlabeled N; bottom figures) N content in new shoots and fine roots protruding from the plugs into the surrounding soil (right figures) and the old organs that mostly originated in the previous growing season (coarse roots, plug fine roots, stems and old leaves) (left figures) of *Quercus coccifera*, *Q. ilex*, *Olea europaea* and *Pinus halepensis* seedlings measured at different growth stages. (t_0): after ^{13}C labeling and before transplanting to spring growth conditions; (t_1): shoot elongation had not yet started but seedlings had produced significant amounts of fine roots protruded the plug; (t_2): first shoot flush of growth had finished and most leaves had completely unfolded and matured. Data are means ±1 SE (n=10).

Supplementary material

Table S1. Two way ANOVA results for species and developmental stage effects on the amount of new (current photosynthesis and soil N) and old (reserves) C and N in different organs of *Quercus coccifera*, *Q. ilex, Olea europaea* and *Pinus halepensis*. Data are F values. Where *: 0.01 < P ≤ 0.05; **: 0.001 < P ≤ 0.01; ***: P ≤ 0.001, ns: P > 0.05; † P = 0.07.

	New shoots	Old leaves	Stems	Coarse roots	Plug fine roots	Protruding fine roots
C old (mg)						
Species (1)	5.1 **	38 ***	112 ***	193 ***	453 ***	2.1 n.s.
Develop. stage (2)		59 ***	6.7 **	12 ***	3828***	105 ***
1 × 2		2.5 *	4.24 ***	3,7 **	429 ***	2.3 ns
C new (mg)						
Species (1)	6.9 **	5.4 **	11 ***	41 ***	8.5 ***	4.1 **
Develop. stage (2)		0.06 ns	6.8 *	6.7 *	0.6 ns	93 ***
1 × 2		3.3 *	3.1 *	1.9 ns	0.23 ns	3.0 *
N old (mg)						
Species (1)	14 ***	26 ***	167 ***	102 ***	110 ***	3.2 *
Develop. stage (2)		2.6 †	3.1 *	0.24 ns	6.8 **	0.09 ns
1 × 2		0.41 ns	2.5 *	0.73 ns	2.4 *	1.5 ns
N new (mg)						
Species (1)	13 ***	16 ***	23 ***	54 ***	41 ***	5.8 **
Develop. stage (2)		58 ***	139 ***	35 ***	106 ***	305 ***
1 × 2		17 ***	6.2 **	6.7 ***	11 ***	5.4 **

Figure 1

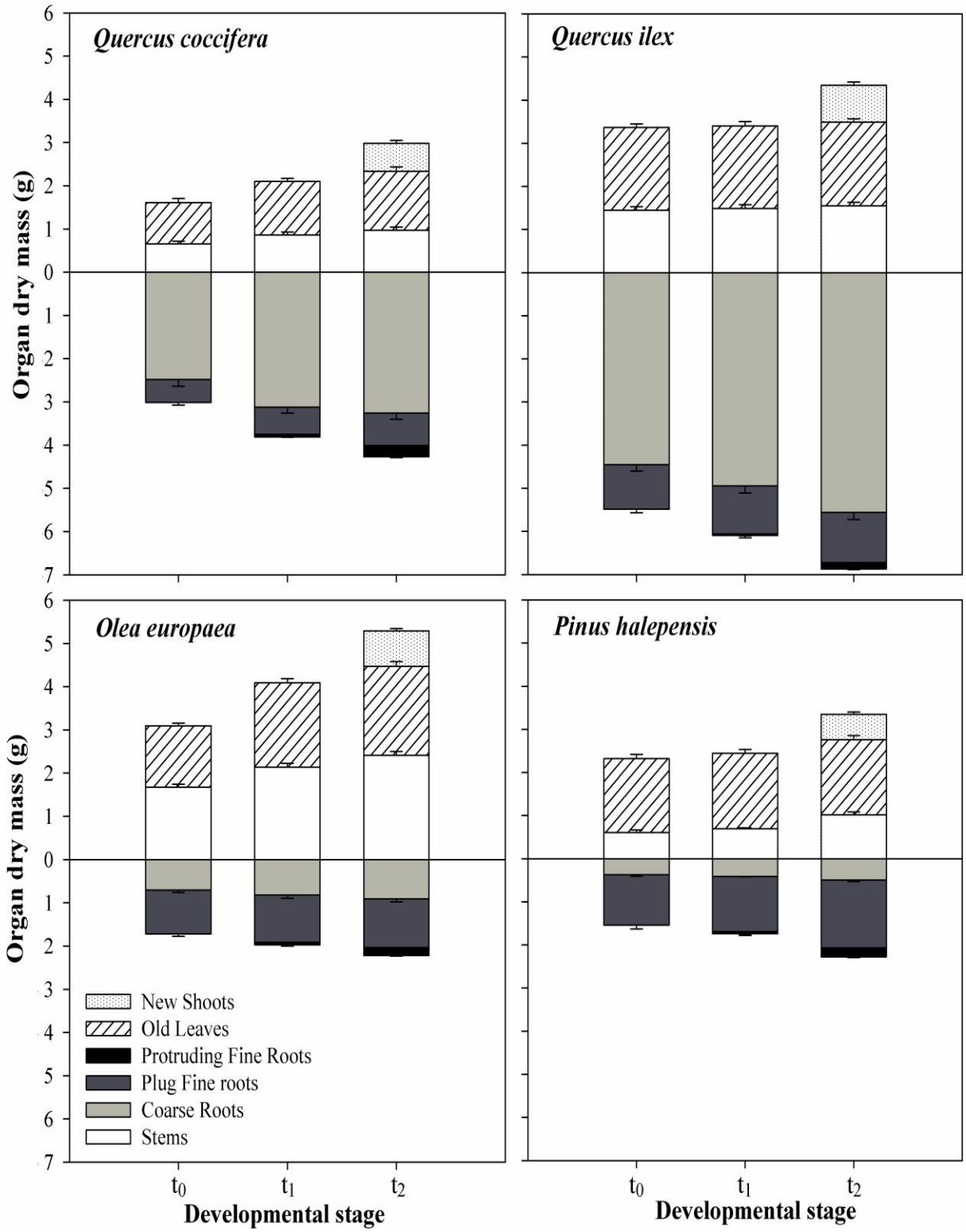

Quercus coccifera
Quercus ilex
Olea europaea
Pinus halepensis
Organ dry mass (g)
Organ dry mass (g)
6 5 4 3 2 1 0 1 2 3 4 5 6 7
New Shoots
Old Leaves
Protruding Fine Roots
Plug Fine roots
Coarse Roots
Stems
t0 t1 t2
Developmental stage
t0 t1 t2
Developmental stage

Figure 2

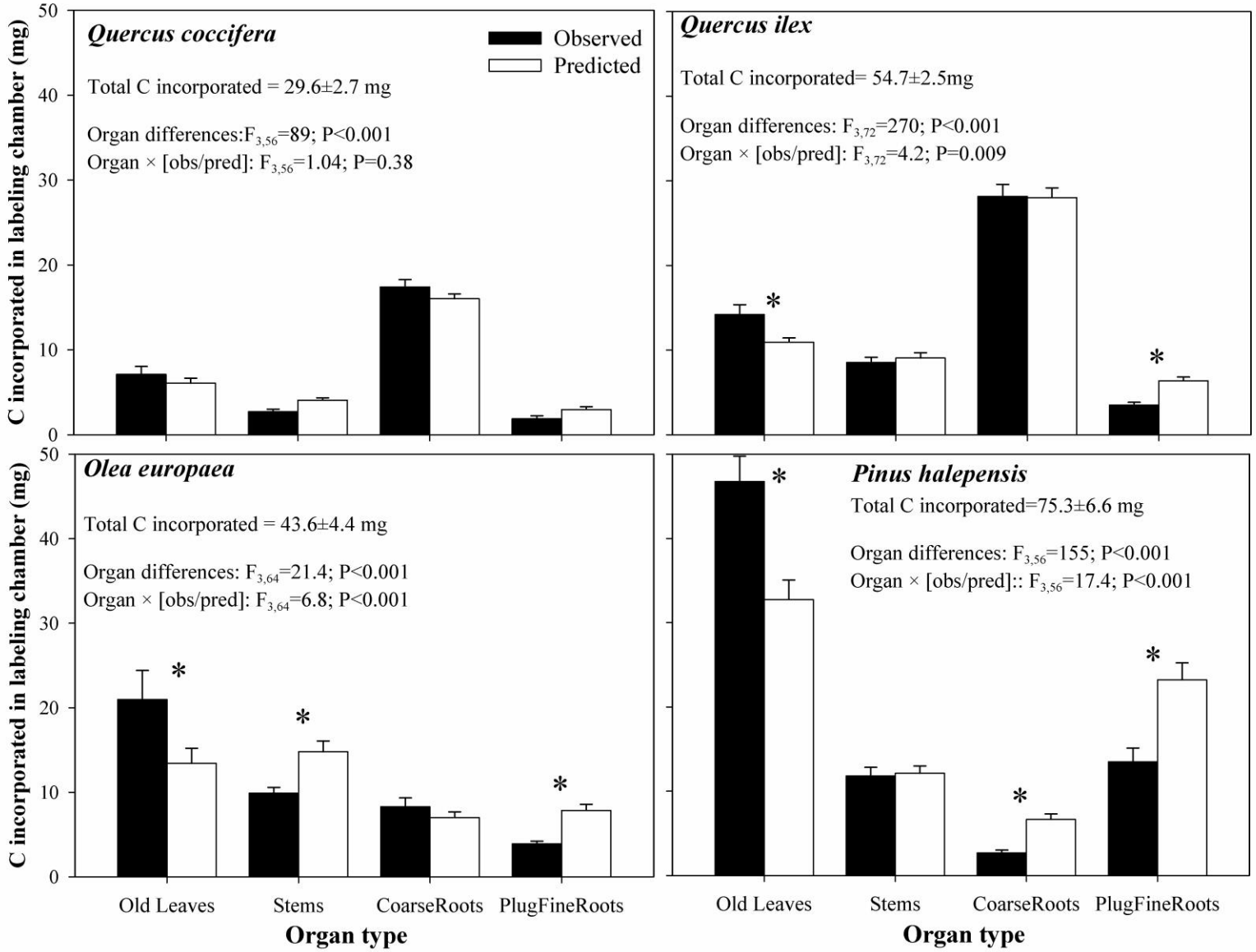

Quercus coccifera
Observed
Predicted
Total C incorporated = 29.6±2.7 mg
Organ differences:F3,56=89; P<0.001
Organ × [obs/pred]: F3,56=1.04; P=0.38
Quercus ilex
Total C incorporated= 54.7±2.5mg
Organ differences: F3,72=270; P<0.001
Organ × [obs/pred]: F3,72=4.2; P=0.009
Olea europaea
Total C incorporated = 43.6±4.4 mg
Organ differences: F3,64=21.4; P<0.001
Organ × [obs/pred]: F3,64=6.8; P<0.001
Pinus halepensis
Total C incorporated=75.3±6.6 mg
Organ differences: F3,56=155; P<0.001
Organ × [obs/pred]:: F3,56=17.4; P<0.001
C incorporated in labeling chamber (mg)
0
10
20
30
40
50
Old Leaves
Stems
CoarseRoots
PlugFineRoots
Organ type

Figure 3

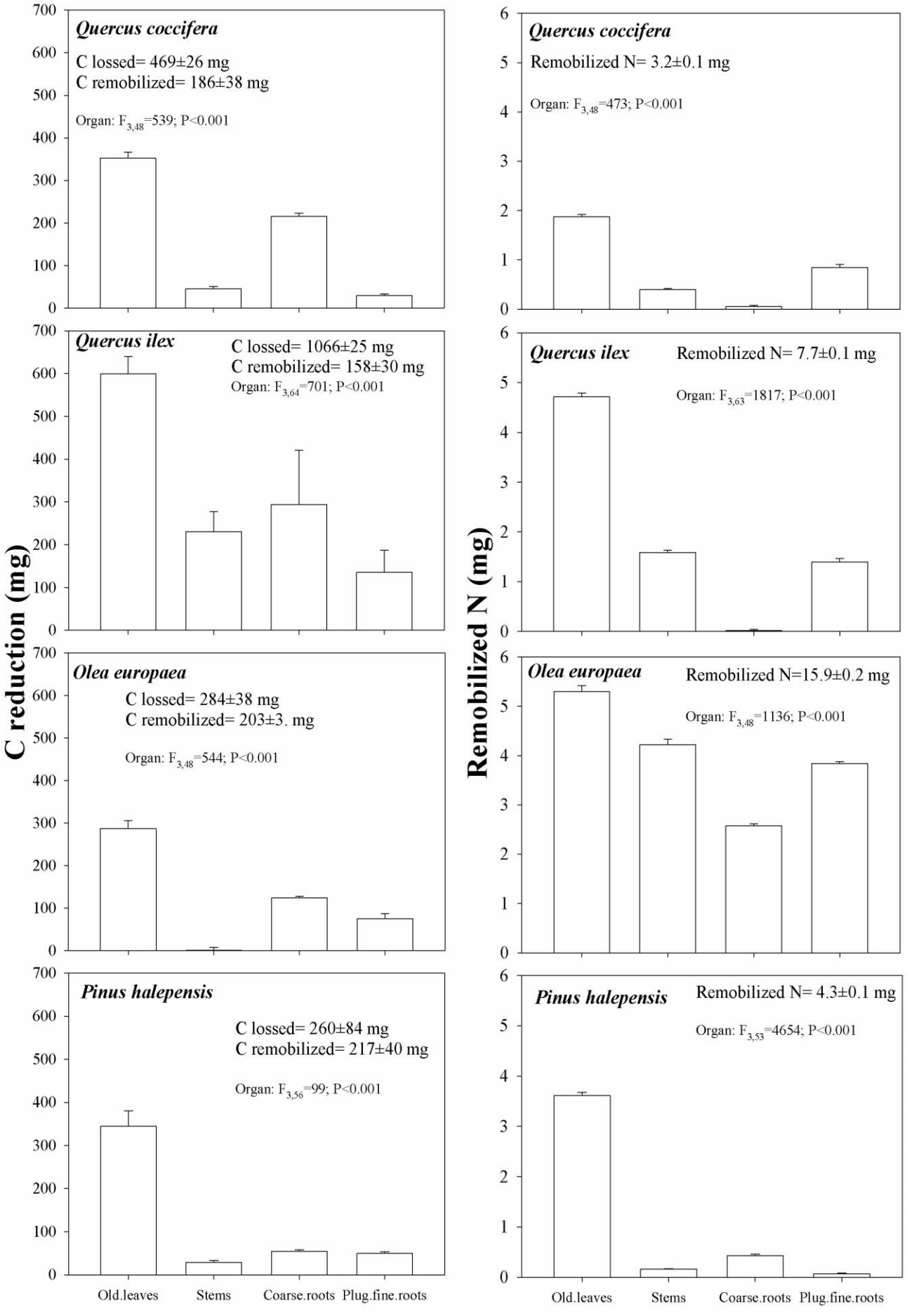

C reduction (mg)
Remobilized N (mg)
Quercus coccifera
C lossed= 469±26 mg
C remobilized= 186±38 mg
Organ: F3,48=539; P<0.001
Quercus coccifera
Remobilized N= 3.2±0.1 mg
Organ: F3,48=473; P<0.001
Quercus ilex
C lossed= 1066±25 mg
C remobilized= 158±30 mg
Organ: F3,64=701; P<0.001
Quercus ilex
Remobilized N= 7.7±0.1 mg
Organ: F3,63=1817; P<0.001
Olea europaea
C lossed= 284±38 mg
C remobilized= 203±3. mg
Organ: F3,48=544; P<0.001
Olea europaea
Remobilized N=15.9±0.2 mg
Organ: F3,48=1136; P<0.001
Pinus halepensis
C lossed= 260±84 mg
C remobilized= 217±40 mg
Organ: F3,56=99; P<0.001
Pinus halepensis
Remobilized N= 4.3±0.1 mg
Organ: F3,53=4654; P<0.001
Old.leaves
Stems
Coarse.roots
Plug.fine.roots

Figure 4

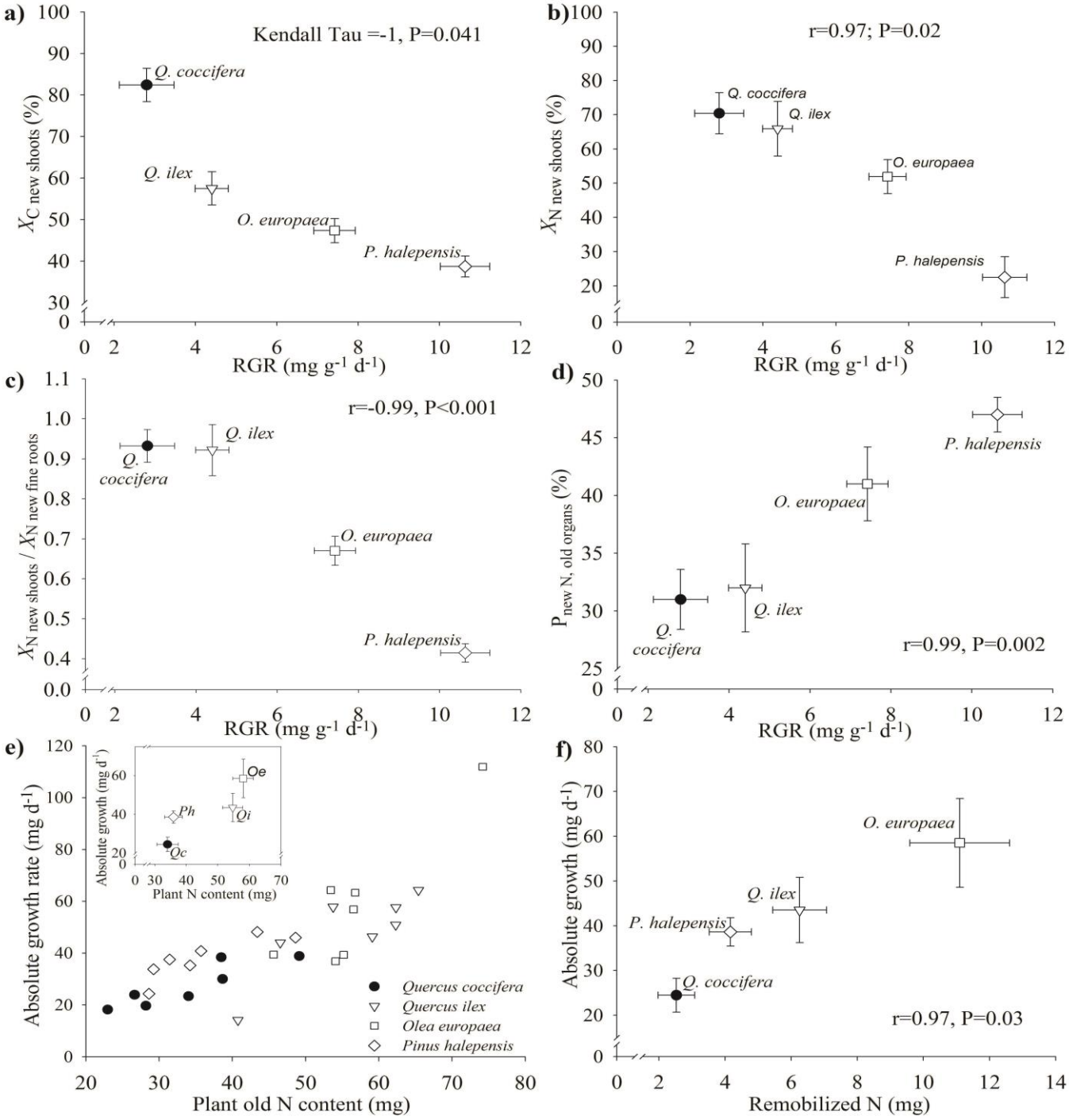

a)
Kendall Tau =-1, P=0.041
X_C new shoots (%)
Q. coccifera
Q. ilex
O. europaea
P. halepensis
RGR (mg g-1 d-1)
b)
r=0.97; P=0.02
X_N new shoots (%)
Q. coccifera
Q. ilex
O. europaea
P. halepensis
RGR (mg g-1 d-1)
c)
r=-0.99, P<0.001
X_N new shoots / X_N new fine roots
Q. coccifera
Q. ilex
O. europaea
P. halepensis
RGR (mg g-1 d-1)
d)
$P_{new\ N,\ old\ organs}$ (%)
P. halepensis
O. europaea
Q. ilex
Q. coccifera
r=0.99, P=0.002
RGR (mg g-1 d-1)
e)
Absolute growth rate (mg d-1)
Absolute growth (mg d-1)
Plant N content (mg)
Oe
Ph
Qi
Qc
Quercus coccifera
Quercus ilex
Olea europaea
Pinus halepensis
Plant old N content (mg)
f)
Absolute growth (mg d-1)
O. europaea
Q. ilex
P. halepensis
Q. coccifera
r=0.97, P=0.03
Remobilized N (mg)

Figure SI

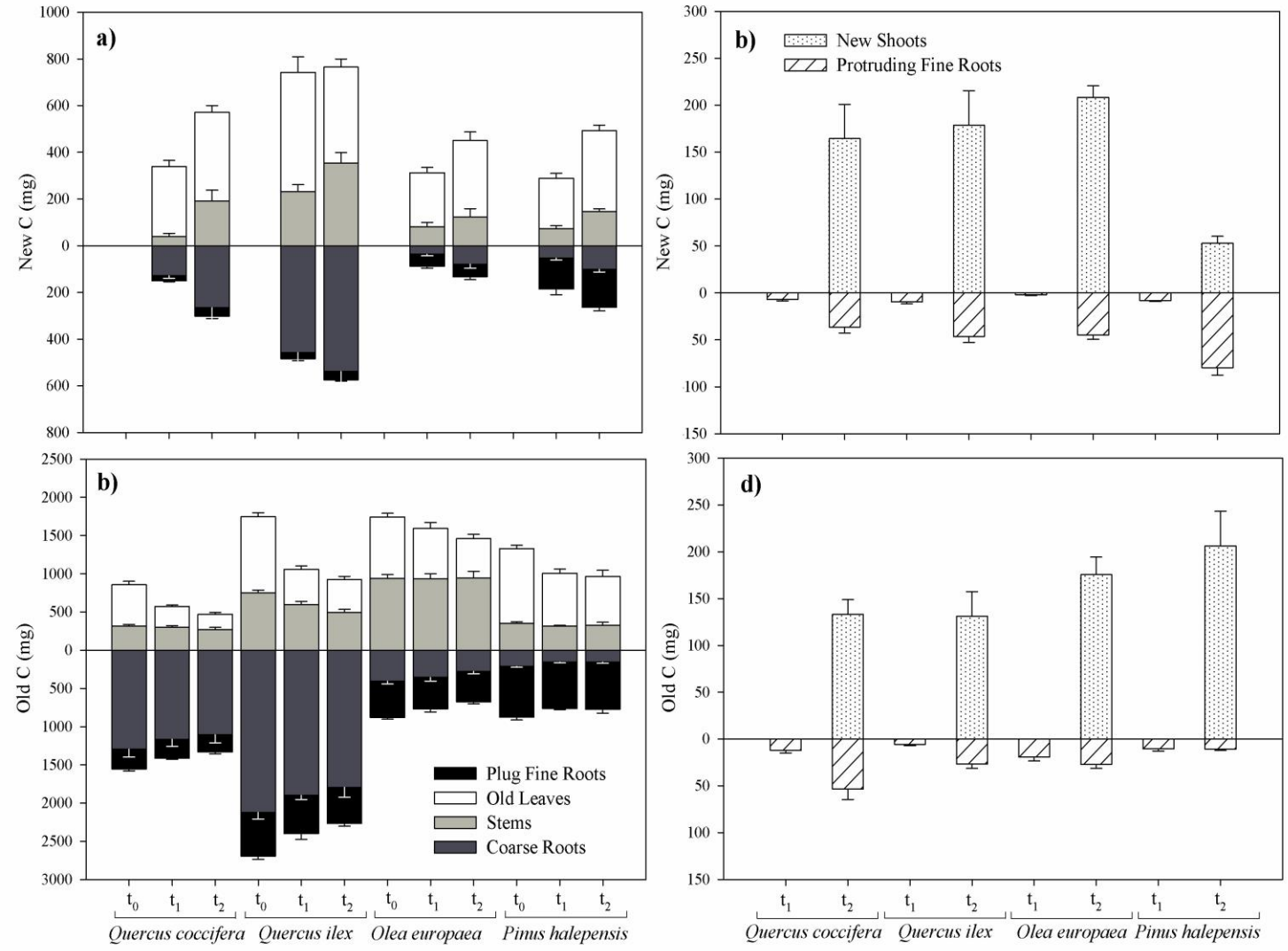
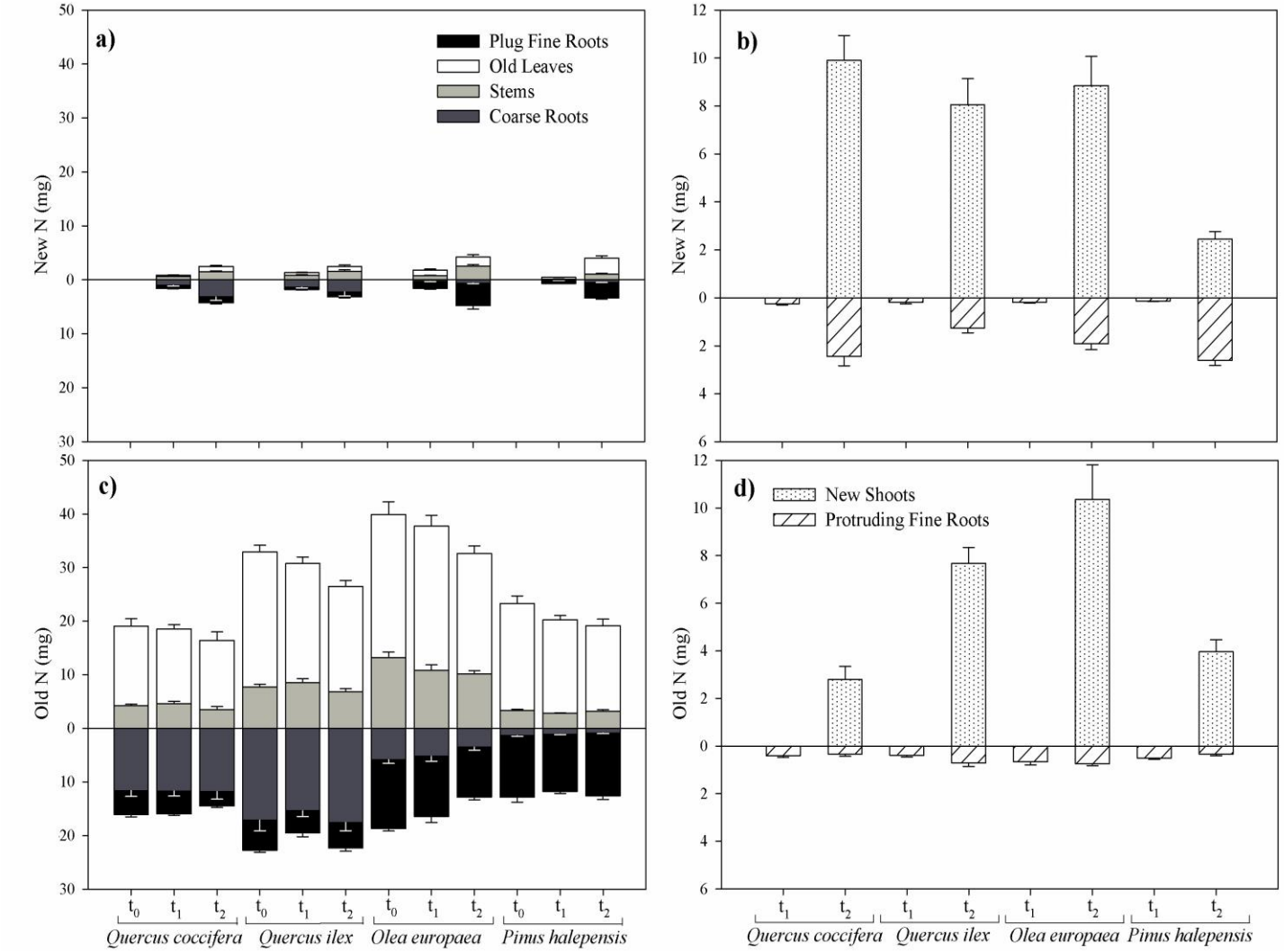

a)
New C (mg)
b)
New Shoots
Protruding Fine Roots
New C (mg)
b)
Old C (mg)
Plug Fine Roots
Old Leaves
Stems
Coarse Roots
d)
Old C (mg)
t0 t1 t2 t0 t1 t2 t0 t1 t2 t0 t1 t2
Quercus coccifera Quercus ilex Olea europaea Pinus halepensis
t1 t2 t1 t2 t1 t2 t1 t2
Quercus coccifera Quercus ilex Olea europaea Pinus halepensis

Figure SII


a)
Plug Fine Roots
Old Leaves
Stems
Coarse Roots
New N (mg)
b)
New N (mg)
c)
Old N (mg)
d)
New Shoots
Protruding Fine Roots
Old N (mg)
t0 t1 t2
Quercus coccifera
Quercus ilex
Olea europaea
Pinus halepensis