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ORIGINAL ARTICLE

Are storage and tree growth related? Seasonal nutrient and carbohydrate dynamics in evergreen and deciduous Mediterranean oaks

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Abstract

Key message **Seasonal dynamics of branch carbohydrates differed sharply between coexisting evergreen and deciduous Mediterranean oaks. Branch carbon storage was crucial in the evergreen, while it played a minor role in the deciduous oaks.**

Abstract The aim of this study was to describe the seasonal dynamics of nutrients and NSC in relation to the aboveground phenology of coexisting winter-deciduous (*Quercus faginea*) and evergreen (*Quercus ilex* subsp. *ballota*) oak species, and to analyse the relationship between the resource budget of branches and shoot growth. Monthly concentrations of nitrogen (N), phosphorus (P), potassium (K) and non-structural carbohydrates (NSC) plus the aboveground phenology of branches were measured over 2 years. We also analysed the correlation between the resource budget of branches prior to the growing season and the subsequent shoot and stem growth. Seasonal branch nutrient dynamics could be explained by shoot growth phenology, showing similar patterns across species. However, NSC dynamics varied between the two species, owing to the differences in leaf phenology and the contrasting role of branches as storage sites. NSC and N branch storage were crucial for the early stages of shoot growth in the evergreen trees. Accordingly, branch N and NSC storage pools in late winter correlated positively with spring growth, and NSC concentrations dropped during bud burst in all branch organs of *Q. ilex*. Contrastingly, branch NSC concentrations of the deciduous *Q. faginea* were only marginally affected by spring growth and no relationship was observed between branch N and NSC stores prior to bud burst. These results challenge previous assumptions on the lower relevance of branch NSC storage for the spring growth of evergreen trees and call for further studies where closely related pairs of coexisting evergreen and deciduous tree species are compared.

Keywords *Quercus ilex* subsp. *ballota* · *Quercus faginea* · Non-structural carbohydrates · Nitrogen · Phosphorus · Seasonal dynamics

Introduction

The accumulation of nutrients (N, P and K) and carbon (C) into storage compounds is a crucial process for the survival and growth of plants (Chapin et al. 1990; Kozlowski 1992; Dietze et al. 2014). On a daily basis, all plants store C into starch during the day, which can be supplied for growth and respiration at night (Smith and Stitt 2007). The storage of C and nutrients has been suggested to play a crucial role in the seasonal growth of trees, particularly in deciduous species, while evergreens are supposed to supply their spring flushing mostly by current photo-assimilates (Hoch et al. 2003; Schädel et al. 2009; Hoch 2015). In long-lived plants like trees, the storage of mobile C compounds and nutrients

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secures their availability when assimilation or uptake decouple from use, promoting plant survival after stress and disturbances (e.g. Canadell and López-Soria 1998; Millard et al. 2001; El Omari et al. 2003; Myers and Kitajima 2007; Hartmann et al. 2013; Piper and Fajardo 2014). The fundamental role of stores in tree survival after stress and disturbance and the forecasted increase in extreme climatic events and disturbances (Kurz et al. 2008; McDowell et al. 2008, 2011; Paritsis and Veblen 2011; Gaylord et al. 2013) have led to a bloom of studies on tree storage, mainly related to C compounds (Dietze et al. 2014). However, despite these recent advances, knowledge on the regulation and dynamism of nutrient and C stores in trees is still limited (Palacio et al. 2014; Hartmann and Trumbore 2016).

One particular aspect that needs attention is the role and regulation of the seasonal variability of C and nutrient stores in different tree functional types and biomes and their relationship to the growth patterns of plants (Martínez-Vilalta et al. 2016). The analysis of the seasonal variability of non-structural carbohydrates (NSC) and nutrient (N, P and K) concentrations in tree organs in relation to environmental conditions and tree phenology may inform about the factors regulating nutrient and C storage (Chapin et al. 1990; Kozlowski 1992; Martínez-Vilalta et al. 2016). Trees living under similar environmental conditions may show different dynamics of C and nutrient storage if they differ in their phenology (Larcher and Thomaser-Thin 1988; Mooney et al. 1992; Barbaroux and Bréda 2002; Newell et al. 2002; Milla et al. 2005; Palacio et al. 2007b). The development of new shoots is one of the largest seasonal sinks for trees and, consequently, variations in shoot growth phenology may have a significant impact on the seasonal patterns of nutrients and C concentrations in tree organs (Kozlowski 1992; Chapin et al. 1990; Klein et al. 2016). Further, given that leaves are the main assimilative organ of plants, and photosynthesis depends on N and P availability (Niinemets et al. 1999), differences in leaf habit might lead to variations in the seasonal course of nutrient and C storage (Newell et al. 2002; Milla et al. 2005).

Several authors have reported differences in the carbohydrate and nutrient dynamics of deciduous and evergreen species in temperate ecosystems (Mooney and Hays 1973; Gray 1983; Hoch et al. 2003; Palacio et al. 2007a, 2014; Schädel et al. 2009). It is generally assumed that evergreen species display less dramatic seasonal fluctuations of NSC reserves than deciduous species (Kozlowski 1992; Schädel et al. 2009). In evergreen species, old foliage may supply newly fixed carbohydrates to growing sinks, lessening the reliance of growth on stored carbohydrates (Hansen and Beck 1994). Consequently, it has been suggested that evergreens are less dependent on both external nutrient uptake and the remobilization of NSC from reserves during spring flushing than deciduous species (Mooney and Hays 1973; Gray 1983; Schädel et al. 2009; Hoch 2015). Further, increased C storage may favour future growth, so that trees (particularly deciduous) with larger C stores may grow more (Chapin et al. 1990; Pérez-de-Lis et al. 2016). Regarding the preferred site of storage, evergreens have been reported to store nutrients (particularly N) preferentially in the old foliage and young twigs, while deciduous species reportedly store nutrients mainly in the roots and trunks (Millard and Grelet 2010). For NSC, the standing view is that stores accumulate throughout the plant, with no preferred sites of storage related to leaf habit (Kozlowski 1992; Millard and Grelet 2010; Hoch 2015).

There are, however, important exceptions to these general assumptions. For example, several studies showed similar amplitude in the seasonal variation between evergreen and deciduous species (Hoch et al. 2003; Palacio et al. 2007a; Martínez-Vilalta et al. 2016), and similar dependence on C stores for spring flushing between evergreen and deciduous species (von Felten et al. 2007). Also, Mediterranean deciduous and evergreen trees were found to accumulate nutrients and mobile carbohydrates preferentially in the young branches (Mooney and Hays 1973; Cherbuy et al. 2001). It should be noted, however, that most previous studies compared evergreen gymnosperms (conifers) with winter-deciduous angiosperms (see reviews by Millard and Grelet 2010; Brüggemann et al. 2011). Such comparison may not be ideal, since both groups of species show a distant evolutionary origin and important differences other than leaf habit (Augusto et al. 2014), which may hamper the extrapolation of results from evergreen gymnosperms to angiosperms. Further, intra-generic comparisons are lacking and data on certain drought-prone biomes like Mediterranean ecosystems are particularly scant (Martínez-Vilalta et al. 2016).

Trees and shrubs growing in Mediterranean ecosystems show a vast diversity of coexisting growth forms and phenological patterns (Orshan 1989). Mixed forests of deciduous and evergreen broadleaved trees, mainly composed by oaks, are widespread in the Mediterranean Basin (Quézel and Médail 2003). These mixed forests offer unique scenarios to explore questions related to the effect of leaf habit on the C and nutrient dynamics of plants avoiding phylogenetic bias. Previous studies have analysed the seasonal nutrient and/or NSC dynamics of individual *Quercus* species including *Q. suber* (Oliveira et al. 1996; Cerasoli et al. 2004), *Q. rubra* (Le thiec et al. 1995), *Q. ilex* (Larcher and Thomaser-Thin 1988; Escudero et al. 1992a; Milla et al. 2005; Sanz-Perez et al. 2009; Rosas et al. 2013; Uscola et al. 2015), *Q. faginea* (Sanz-Pérez et al. 2009), *Q. petraea* (Barbaroux and Bréda 2002; El Zein et al. 2011; Klein et al. 2016), *Q. pyrenaica* (Salomón et al. 2016; Pérez-de-Lis et al. 2017), *Q. robur* (Pérez-de-Lis et al. 2017) or *Q. coccifera* (Diamantoglou and Meletiou-Christou 1978; Diamantoglou and Kull 1988; Meletiou-Christou et al. 1994; Milla et al. 2005). However, studies including natural populations of co-existing mature oaks with different leaf habit have, to the best of our knowledge, not been conducted to date.

The aim of this study was to describe the seasonal dynamics of nutrients (N, P and K) and NSC in relation to the aboveground phenology of coexisting oak trees of the winter-deciduous species *Q. faginea* and the evergreen *Q. ilex* subsp. *ballota*, and to analyse the relationship between the resource budget of the branches of individual trees at different stages of the growth season and the subsequent shoot and stem growth. Our hypotheses were that: (1) the seasonal dynamics of nutrient and NSC in the branches of both study species will be explained by the phenology of shoot growth and leaf senescence; (2) the winter deciduous species will show sharper variations in the seasonal N and NSC stores than the evergreen, with minimum values at the beginning of shoot growth (i.e. higher reliance on storage); (3) trees, particularly of the winter deciduous species, with larger C and N storage pools in the branches at the beginning of the growing season will also show increased branch and stem growth.

Materials and methods

Study species and sites

The study site is located in Agüero, Huesca province, Aragón, northeastern Spain (42°18′N, 0°47′W, 750 m a.s.l.). Climate is Mediterranean and continental, being characterized by dry summers and cold winters with 631 mm and 13.8 °C total annual precipitation and mean annual temperature, respectively (Ayerbe meteorological station, 42°16′N, 0°41′W, 585 m a.s.l., located at ca. 10 km from the study site; see Online Resource 1 Fig. S1). Soil in this area is Calcisol (FAO 1998), formed on Miocene clays with bedrock of calcareous sandstone. Vegetation is an open woodland with many low trees, dominated by *Q. ilex* subsp. *rotundifolia, Q. faginea, Arbutus unedo* L. and *Pinus halepensis* Mill., approximately with similar dominance, and other less abundant woody species. For further details on the study site see Montserrat-Martí et al. (2009).

Quercus ilex subsp. *ballota* (from here on, *Q. ilex*) is an evergreen oak tree that in the Iberian Peninsula grows preferentially inland under continental conditions (Amaral Franco 1990). In the study area, this species usually forms shrubby-type crowns (Alla et al. 2013). *Q. faginea* is a deciduous oak tree with tree-like crowns and a wide distribution in sub-Mediterranean areas, mainly in the Iberian Peninsula (Amaral Franco 1990). Both species coexist in the study area forming secondary forests composed of multi-stemmed trees of similar age, but *Q. faginea* individuals are taller (see Online Resource 1 Table S1) than *Q. ilex* ones (Alla et al. 2013). In the study site, both species show determinate growth, displaying one sole pulse of growth in spring, while they differ sharply in their average leaf longevity, with leaves of *Q. ilex* living up to 4 years and those of *Q. faginea* living up to 8 months (Montserrat-Martí, unpublished data).

Phenological measurements

Phenological patterns were obtained for 15 individuals per species in 2006 and 2007, although only results for the five trees of each species included in the analysis of the seasonality of nutrient and NSC concentrations (see details in Online Resource 1 Table S1) are shown. The following phenophases were considered in both species: BS = bud swelling, BB = bud burst (buds were open and leaves or inflorescences were partially shown, but stems were not visible yet), SG = shoot growth (from the time when new stems were visible within bursting buds to the end of leaf and stem extension), SBF = scaled bud formation (at the end of shoot growth apical hypsophillary buds develop scaled buds, see Nitta and Ohsawa 1998), FBF = flower bud formation, F = flowering, FS = fruit setting, SD = seed dispersal, LS = leaf shedding. The percentage of each phenophase was estimated in the canopy of each individual according to Montserrat-Martí et al. (2009). Only those phenophases that occurred in more than 5% of the branches of the tree's canopy are shown in figures. In the case of LS, we only considered senescent and dead leaves that were close to being shed: in the deciduous species when they represented more than 5% of all leaves, and in the evergreen when they were more than 5% of the old leaves. Marcescent leaves (dry leaves retained in the canopy), which are frequent in *Q. faginea*, were not considered in LS but in a different phenophase not shown in the results. For more information about SD and other details on the phenology of these species in the study location, see Montserrat-Martí et al. (2009).

Experimental design

The study was performed in two stages: first, we analysed the seasonal variability in nutrient (N, P, K) and NSC mass-based concentrations of three-year-old branches of the two study species; second, we focused on the specific relationship between N and NSC storage (measured both as concentrations and 3-year-old branch pools) and the primary and secondary growth of trees. Both approaches required different sampling designs.

Plant harvest for the analysis of seasonal nutrient and carbohydrate concentrations

To analyse the seasonal change in nutrient and NSC concentrations, we collected 3-year-old branches from five tagged individuals of *Q. ilex* and *Q. faginea* growing at the study site (see average characteristics of study trees in Online Resource 1, Table S1). The age of branches was determined by counting the scars left by bud scales at the beginning of each pulse of shoot growth. Trees were sampled monthly, fortnightly during spring shoot growth, over two consecutive years (between January 2006 and December 2007). Between 4 and 15 3-year-old branches from middle sunlit positions within the mid-third of the crown were collected from study individuals. The collection of these branches accounted for a small percentage of the canopy of the tree and had no noticeable effects on growth or phenology. Branches were placed in a cooler and immediately taken to the laboratory for fractionation and storage. Once in the laboratory, branches were separated in the following fractions (when available): current-year leaves and stems, 1- and 2-year-old stems, 1- and 2-year-old leaves (just in *Q. ilex*), flowers, fruits and buds. Samples for NSC and N analyses were analysed separately for each individual, while analyses of K and P concentrations were conducted on a composite sample obtained by pooling material from the five individuals sampled on each date. All samples were freeze-dried and milled in a ball mill (Retsch M400, Haan, Germany) to a fine powder prior to analyses.

The seasonal amplitude of N, starch and NSC concentrations (Diff) was calculated as relative variation (%) from the average seasonal maximum between 2006 and 2007 (i.e. $\text{Diff} = 100 \times (\text{Max} - \text{Min})/\text{Max}$), where Max and Min are the average seasonal maximum and minimum values, respectively, recorded during the 2 years of study.

Plant harvest for the study of the relationship between N and NSC pools and growth

To evaluate the relationship between N and NSC storage and tree growth in the study species, we harvested a minimum of 20 2-year-old branches from 10 to 11 tagged individuals of *Q. faginea* and *Q. ilex*, respectively. Branches were collected from middle sunlit positions within the mid-third of the crown and were selected to represent the average size of branches of each tree. The collection of these branches accounted for a small percentage of the canopy of the tree (Online Resource 1, Table S1), with no noticeable effects on growth or phenology. To gain information of the storage levels before the onset of shoot growth, branches were collected in February 2012, when trees were still in a resting winter stage (i.e. without swollen buds). To detect the effect of spring elongation growth on stores, branches were collected when the extension of most shoots was completed, but stems and leaves were still young and tender (approximately 2 weeks after the beginning of bud burst, i.e. April in the case of *Q. faginea* and May in the case of *Q. ilex*). This aimed at capturing the heterotrophic phase of shoot elongation, when the drain on stores is supposed to be larger. Finally, to capture storage levels at the end of the shoot growth season, both species were sampled in July, when shoots were already mature. Once in the laboratory, branches were separated in the following fractions (when available): current-year leaves and stems, 1-year-old stems, 1-year-old leaves (just in *Q. ilex*), flowers and fruits. All samples were freeze-dried, weighed and subsequently milled in a ball mill (Retsch M400, Haan, Germany) to a fine powder prior to chemical analyses.

Pools were calculated as the product between the mass-based concentrations and the biomass of the different fractions. Pools of vegetative fractions included in 2-year-old branches were subsequently added up to calculate N and NSC pools per 2-year-old branch in the three different sampling times (i.e. before bud burst, right after the completion of shoot elongation and at the end of shoot growth). The variation in the amount of starch, NSC and N in the vegetative components of branches after shoot growth ($\Delta\text{starch}_{\text{pool}}$, $\Delta\text{NSC}_{\text{pool}}$ and $\Delta\text{N}_{\text{pool}}$, respectively) was calculated as the difference between pools of branches collected in winter and spring. This variation was also calculated taking into account the change in starch, NSC and N storage pools of source fractions ($\Delta\text{starch}_{\text{pool sources}}$, $\Delta\text{NSC}_{\text{pool sources}}$ and $\Delta\text{N}_{\text{pool sources}}$, respectively) of the branch only, i.e. old stems in the case of *Q. faginea* and old leaves and stems in *Q. ilex*.

Growth measurements

Growth measurements were taken on the same 10 and 11 tagged individuals of *Q. faginea* and *Q. ilex*, respectively, used for branch pool calculation in 2012. The absolute shoot growth of branches in spring ($\text{ASG}_{\text{spring}}$) was calculated as the vegetative biomass increment of the average weight of the 2-year-old branches sampled for N and NSC analyses between winter ($\text{BM}_{\text{Winter}}$, measured in February, before shoot growth) and spring ($\text{BM}_{\text{Spring}}$, measured in April and May matching the end of extension growth in *Q. faginea* and *Q. ilex*, respectively).

$$(\text{ASG}_{\text{Spring}}) = \text{BM}_{\text{Spring}} - \text{BM}_{\text{Winter}}. \tag{1}$$

Due to the irregular and low reproductive production of the studied populations, calculations were based only on vegetative branch components. To account for potential differences due to the variation in the size of branches, the relative primary growth in spring ($\text{RSG}_{\text{Spring}}$) was also calculated as the increment in vegetative biomass of 2-year-old branches between winter ($\text{BM}_{\text{Winter}}$) and spring ($\text{BM}_{\text{Spring}}$) in relation to the total biomass of branches in spring:

$$\text{RSG}_{\text{spring}} = 100 \times \text{ASG}_{\text{Spring}} / \text{BM}_{\text{Spring}}. \tag{2}$$

To calculate $\text{ASG}_{\text{Spring}}$ and $\text{RSG}_{\text{Spring}}$, the biomass of shoots was measured in April in *Q. faginea* and in May in

Q. ilex to account for the aforementioned differences in the timing of bud burst between both species.

Radial growth was measured using manual band dendrometers (Agriculture Electronics Corporation, Tucson, USA) during the two study years in the same individuals used for branch pool calculation in 2012. In early 2006, dendrometers were placed at 1.3 m on the thickest stem of each individual. Dead bark was brushed off before installing the dendrometers, which were read at 24-day intervals on average. The displacements of the dendrometers (cumulative perimetrical increment) were measured annually, from their installation in 2006 to the end of the study in 2012, with a precision of 0.1 mm. To estimate annual radial growth, March (before radial growth started; cf. Albuixech et al. 2012) and December (when growth finished) dendrometer readings were converted to cumulative radial growth (mm) or to basal area increment (mm^2) by assuming a circular stem shape. Data recorded in 2012 were used for the analysis of the relationship between storage pools and growth.

Chemical analyses

Total N mass-based concentrations were analysed with an elemental analyser (Elementar VarioMAX N/CM, Hanau, Germany), P concentration was assessed by vanado-molybdate colorimetry (Allen 1989) and K content was measured with a flame photometer. Soluble sugars (SS) were extracted with 80% (v/v) ethanol in a water bath at 60 °C, and their concentration determined colorimetrically, using the phenol–sulphuric method of Dubois et al. (1956) as modified by Buysse and Merckx (1993). Starch and complex sugars remaining in the undissolved pellet after ethanol extractions were enzymatically reduced to glucose using amyloglucosidase (0.5% amyloglucosidase 73.8 U/mg, Fluka 10115) and analysed as described in Palacio et al. (2007a). Non-structural carbohydrates measured after ethanol extraction are referred to as soluble sugars, and carbohydrates measured after enzymatic digestion are referred to as starch. Both are expressed in glucose equivalents. The sum of SS and starch is referred to as total non-structural carbohydrates (NSC).

Statistical analyses

Pearson correlation coefficients were used to analyse the associations between storage (N, NSC and starch concentrations and pools in leaves, stems and the whole branch) and growth variables (ASG$_{\text{spring}}$, RSG$_{\text{spring}}$, annual radial growth and annual basal area increment in 2012, Online Resource 1 Table S4) in each study species. Differences in seasonal N, starch and NSC concentrations between species were explored separately for leaves and stems by generalized linear mixed models (GLMMs) with “species”, “date”, “organ” and “cohort” (i.e. age of the different organs) as fixed factors and “individual tree” as a random factor to account for potential auto-correlative effects of repetitive measures. Simplified models (without the “organ”, “species” and “cohort” factors) were subsequently run for each organ, species and cohort separately to account for variability within each factor level. Residuals were checked for independence, normality and homoscedasticity. N concentrations were log-transformed to meet these requirements. Tukey HSD post hoc tests were run to compare the mean values between dates. Statistical analyses were carried out using JMP 10.0.2 (SAS 9.3 Foundation).

Results

Seasonal dynamics of nutrients (N, P and K)

Both species showed generally similar seasonal dynamics of N, P and K in their leaves and stems, reaching maximum concentrations in newly formed leaves and stems in spring, which were subsequently diluted as organs matured (Figs. 1, 2). Despite such dilution, K concentrations remained relatively high during late spring and summer in all the branch components of both species (Online Resource 1 Fig. S2). Similar to newly formed fractions, N concentrations of 1-year-old stems peaked in both species prior to shoot growth (Fig. 2, Online Resource 1 Fig. S3). N concentrations increased significantly in most of the branch components of both species between summer and autumn ($P < 0.05$), except for the leaves of *Q. faginea*, in which concentrations decreased significantly ($P < 0.05$) during leaf senescence in autumn (Figs. 1, 2). Nevertheless, despite these general similarities, some differences were observed and the interaction between “species” and “date” was significant both for stem and leaf N concentrations (Table 1). Seasonal patterns of *Q. ilex* approximately lagged 1 month behind those of *Q. faginea*, following the differences in shoot growth phenology between species (Figs. 1, 2). Also, P concentrations in branches of *Q. faginea* increased in winter and remained high until bud burst, while P concentrations of *Q. ilex* remained low during winter and only increased with bud swelling (Fig. 1).

Seasonal patterns of nutrient concentrations were generally similar across different leaf and stem cohorts in both species (Online Resource 1, Fig. S2). Consequently, the seasonal pattern of nutrient concentrations of some cohorts that were not growing was similar to that of new expanding cohorts. For example, spring increases in N, P and K concentrations were observed in old cohorts of stems (and in *Q. ilex* also in old leaf cohorts) during bud swelling and bud burst (Figs. 1, 2; Online Resource 1, Fig. S2).

Nutrient concentrations showed similar seasonal variation in the 2 years studied (Figs. 1, 2). However, lower

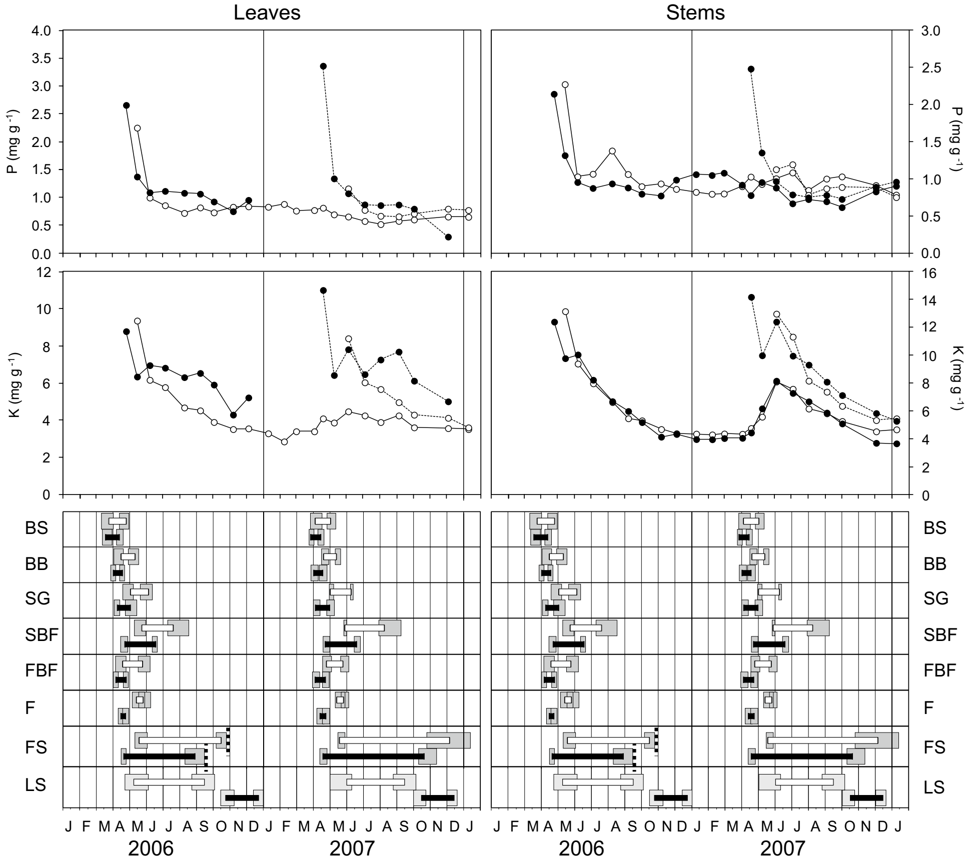

Fig. 1 Upper panels: seasonal dynamics of P and K concentrations in 2006 (solid lines) and 2007 (broken lines) leaves (left panel) and stems (right panel) of *Quercus ilex* (white circles) and *Quercus faginea* (black circles) throughout the two study years (2006 and 2007). Values are composite samples obtained by pooling material from several individuals within the population. Lower panels: aboveground phenology of *Q. ilex* (white lines) and *Q. faginea* (black lines). Black or white bars indicate periods when the five individuals of each species monitored showed a given phenophase, while grey rectangles indicate variability among study trees. Dotted lines indicate fruit abortion in 2006. *BS* bud swelling, *BB* bud burst, *SG* shoot growth, *SBF* scaled bud formation, *FBF* flower bud formation, *F* flowering, *FS* fruit set

N concentrations were detected in 2007 for all the old leaf and stem cohorts of *Q. ilex* and also in the oldest stems of *Q. faginea* (Fig. 2, Table S3). Also, N and P concentrations of the newly formed leaves and stems of *Q. ilex* in spring were lower in 2007 than in 2006 (Figs. 1, 2), which may be due to differences in the development of branches at sampling. In 2006, new leaves and stems were sampled soon after the beginning of shoot growth, while in 2007 samples were gathered towards the end of this phenophase, when initial N and P concentrations were already diluted. This did not affect K concentrations in the new organs, which showed similarly high spring concentrations in both years (Fig. 1).

Leaves of the winter deciduous species tended to show higher N, P and K concentrations than leaves of the evergreen, while stem concentrations of all nutrients were similar across species (Table 1 for N; Figs. 1, 2). In both species, leaves had higher N than stems ($F = 217.2$, $P < 0.001$), while the opposite was true for K and P. Finally, both species showed progressively lower N, P and K concentrations in successively older leaf and stem cohorts (Online Resource 1, Fig. S3; Table 1 for N).

Seasonal dynamics of non-structural carbohydrates

Seasonal patterns of NSC were different between species both in the leaves and stems (Table 1), while differences between the 2 years studied were small (Fig. 2; Online Resource 1, Fig. S3, Tables S2 and S3). *Q. ilex* showed maximum NSC and starch concentrations at bud burst in spring and minimum values in summer, right after the completion of shoot elongation and leaf senescence (Fig. 2; Online Resource 1, Fig. S3). Differences between spring and summer starch and NSC concentrations were significant for all the cohorts of leaves and stems studied ($P < 0.05$), except for leaves formed in 2004. Carbohydrate concentrations of this species started rising again at the end of summer and did not recover maximum values until late winter (early spring in the case of starch). Patterns were very similar for leaves and stems; although leaves of *Q. ilex* recovered NSC concentrations after bud break sooner than the rest of the cohorts, peaking at the end of summer (September). Also, contrary to what we observed with nutrient concentrations, leaves and stems of the evergreen showed increasing NSC ($F = 130.8$, $P < 0.001$) and starch ($F = 39.7$, $P < 0.001$) concentrations

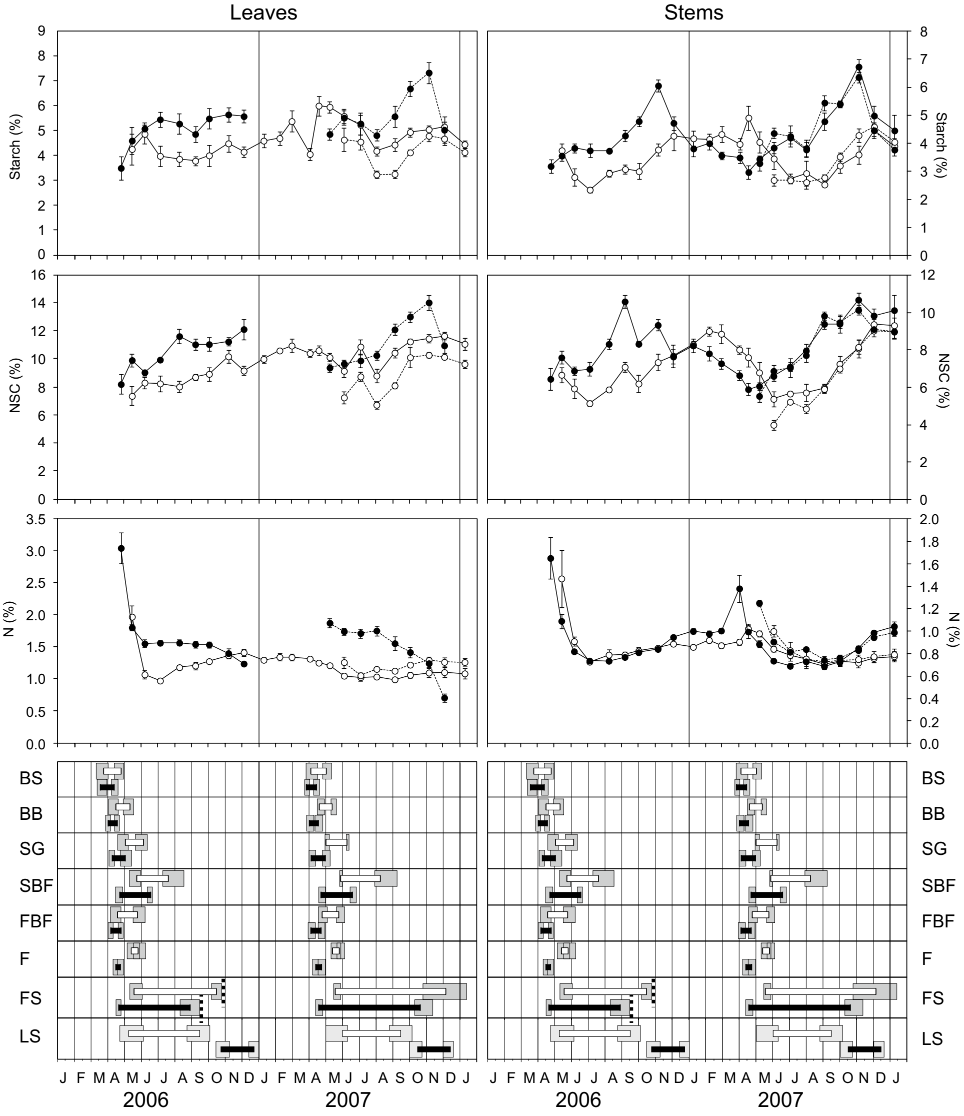

Fig. 2 Upper panels: seasonal dynamics of starch, total non-structural carbohydrates (NSC) and N in 2006 (solid lines) and 2007 (broken lines) leaves (left pannels) and stems (right pannels) of *Quercus ilex* (white circles) and *Quercus faginea* (black circles) throughout the two study years (2006 and 2007). Values are means ± SE, *N* = 5 trees in each species. Lower panels: aboveground phenology of *Q. ilex* (white lines) and *Q. faginea* (black lines). Black or white bars indicate periods when the five individuals of each species monitored showed a given phenophase, while grey rectangles indicate variability among study trees. Dotted lines indicate fruit abortion in 2006. *BS* bud swelling, *BB* bud burst, *SG* shoot growth, *SBF* scaled bud formation, *FBF* flower bud formation, *F* flowering, *FS* fruit set

Table 1 Results of GLMMs on the differences in the nitrogen (N), starch and total non-structural carbohydrate (NSC) concentrations of the leaves and stems of the study species

Factor	*N*		Starch		NSC	
	Leaves	Stems	Leaves	Stems	Leaves	Stems
Species	**13.5 (0.007)**	0.3 (0.605)	1.6 (0.253)	0.1 (0.806)	2.2 (0.175)	0.1 (0.753)
Date	**194.0 (< 0.001)**	**137.2 (< 0.001)**	**4.1 (0.044)**	**30.9 (< 0.001)**	**15.8 (< 0.001)**	0.08 (0.774)
Species × date	**163.7 (< 0.001)**	**23.5 (< 0.001)**	**13.1 (< 0.001)**	**205.1 (< 0.001)**	**16.6 (< 0.001)**	**134.2 (< 0.001)**
Cohort	0.1 (0.775)	**33.2 (< 0.001)**	0.2 (0.673)	**6.5 (< 0.001)**	0.2 (0.680)	**6.9 (< 0.001)**

Models included “tree” as a random factor and “species”, “date”, “cohort” and the interaction between “species” and “date” as fixed factors. Results show *F* ratios and *P* values (in parentheses). Significant effects are highlighted in bold. *N* = 5

with age (as indicated by differences among successively older cohorts, Online Resource 1, Fig. S3).

Contrastingly, carbohydrate concentrations of *Q. faginea* peaked in late autumn–early winter and decreased steadily hereafter, reaching minimal values in spring in the stems and in summer in the leaves (Fig. 2; Online Resource 1, Fig. S3). Different from *Q. ilex*, the decrease in NSC and starch concentrations of the old stems at the beginning of shoot growth was fast and only significant between March and April ($P < 0.05$), matching the short period of bud swelling, while differences between bud swelling (April) and bud burst (May) were not significant ($P > 0.05$) in any of the years of study (Fig. 2; Online Resource 1, Fig. S3). Starch concentrations remained steady during summer in the stems, while they increased significantly during early summer in the newly formed leaves and decreased in mid-summer (Fig. 2; Online Resource 1, Fig. S3). Stem carbohydrate concentrations increased steadily during autumn, reaching the highest values in October–November (Fig. 2; Online Resource 1, Fig. S3). Starch concentrations of all branch organs of the deciduous species showed a sharp decrease during winter matching leaf senescence (Fig. 2; Online Resource 1, Fig. S3). In the stems, such decrease was only partly followed by a reduction in NSC concentrations, indicating it was somewhat due to the inter-conversion of starch into SS. Contrastingly, starch and NSC concentrations in the evergreen species increased steadily during autumn, remained more or less stable during winter and declined in spring (Fig. 2). Starch, NSC and nutrient stores were never fully depleted in any of the species studied (Fig. 2; Online Resource 1, Fig. S3).

Differences between species in the NSC and starch concentrations of the leaves and stems were not significant when all seasonal data were pooled together (Table 1). However, leaves and stems of *Q. faginea* showed higher starch and NSC concentrations than the evergreen species during summer and autumn, while the opposite was true in spring (Fig. 2; Online Resource 1, Fig. S3).

The amplitude of the seasonal variability in starch and NSC concentrations of branch elements was similar between species (Table 2). Contrastingly, N concentrations varied more sharply throughout the growing season in the winter-deciduous species than in the evergreen, both in stems and leaves, except for the cohorts formed in 2006 (Table 2).

Relationship between NSC and N stores in branches and the growth of individual trees

Spring growth (measured both as absolute and relative biomass gain per branch) was not correlated to carbohydrate and N storage pools and concentrations in branches prior to the beginning of the growing season (February) in *Q. faginea* (Table 3). Contrastingly, we detected a significant positive correlation between the N concentrations in the old leaves and the N and NSC pools of *Q. ilex* measured in February (before the beginning of spring growth) and the biomass gain of branches in spring (Table 4).

NSC concentrations in the new leaves and N and carbohydrate branch storage pools in spring were, however, highly correlated to spring growth in *Q. faginea* (Table 3). This indicates that branches of the winter-deciduous trees that grew more were also able to accumulate more NSC in the new leaves early in the season. The opposite pattern was found in *Q. ilex*, where NSC concentrations of stems correlated negatively with branch growth in spring (Table 4). Consequently, branches of the evergreen trees that grew more in spring showed lower NSC concentrations in the stems. The variation in branch storage pools during shoot growth was also positively correlated to branch growth in *Q. ilex* (Table 4), indicating that branches that had larger stores at the beginning of the growing season and remobilized more stores to supply the earlier stages of shoot growth also grew more in spring, and the opposite was indicated for the deciduous trees (Table 3).

Regarding the relationship between branch N and carbohydrate stores and growth at the end of the shoot growing season, leaf NSC concentrations and whole-branch NSC, starch and N storage pools in July were positively correlated to the spring biomass growth of branches in the deciduous trees (Table 3). This indicates that, in *Q. faginea*, branches that grew more in spring were also able to accumulate more NSC in the new leaves and more carbohydrate and N stores in the whole branch at the end of the growing season. No significant correlations were observed between N and carbohydrate stores and primary growth at the end of the growing season in *Q. ilex*.

Finally, in relation to the interplay between storage and secondary growth, our results indicate a significant negative correlation between the N concentrations of old stems from 3-year-old branches in July and the accumulated

Table 2 Seasonal variation (calculated as percentage of the seasonal maximum, %) in the starch, total non-structural carbohydrate (NSC) and nitrogen (N) concentrations of the different cohorts of leaves (L) and stems (S) analysed in the 3-year-old branches of *Q. faginea* and *Q. ilex* trees

Cohorts (organ—year)	*Q. faginea*			*Q. ilex*		
	Starch	NSC	N	Starch	NSC	N
L—2007	34.3	33.1	62.4	32.4	34.5	18.5
L—2006	38.0	32.4	59.4	36.6	36.8	50.8
L—2005	–	–	–	56.0	27.5	35.1
L—2004	–	–	–	51.3	14.8	36.2
S—2007	48.3	45.4	40.6	43.2	55.8	27.3
S—2006	55.9	44.8	58.4	52.2	45.1	51.8
S—2005	53.8	46.8	45.0	59.4	45.1	26.8
S—2004	56.5	53.1	43.7	59.4	44.6	22.3

Table 3 Correlations between growth and storage variables in 3-year-old branches of *Quercus faginea*

Storage variables	Growth variables			
	ASG_{spring} (mg)	RSG_{spring} (% total Apr. branch BM)	Annual radial growth (mm)	Annual basal area increment (mm^2)
NSC leaves Apr (%)	0.632 (0.050)			
N leaves Apr (%)		−0.693 (0.026)		
Starch pool Apr (mg branch^{-1})	0.931 (<0.001)			
NSC pool Apr (mg branch^{-1})	0.943 (<0.001)			
N pool Apr (mg branch^{-1})	0.974 (<0.001)			
$\Delta Starch_{pool}$ (mg branch^{-1})	−0.928 (<0.001)			
ΔNSC_{pool} (mg branch^{-1})	−0.945 (<0.001)			
ΔN_{pool} (mg branch^{-1})	−0.977 (<0.001)			
N stems Jul (%)			−0.806 (0.005)	−0.729 (0.017)
Starch leaves July (%)	0.732 (0.016)			
NSC leaves Jul (%)	0.664 (0.036)			
Starch pool Jul (mg branch^{-1})	0.796 (0.006)			
NSC pool Jul (mg branch^{-1})	0.779 (0.008)			
N pool Jul (mg branch^{-1})	0.760 (0.011)			

Only storage variables with significant correlations are shown. Pearson correlation coefficients along with *P* values (in parentheses) are shown only for significant correlations ($P<0.05$). $N=10$ trees

Storage variables: NSC leaves Apr, N leaves Apr = leaf NSC and N concentrations (respectively) in April; Starch pool Apr, NSC pool Apr, N pool Apr = starch, NSC and N pools (respectively) in 3-year-old branches sampled in April; $\Delta Starch_{pool}$, ΔNSC_{pool}, ΔN_{pool} = difference in the starch, NSC and N pools (respectively) of 3-year-old branches sampled in February and April; N stems Jul = N concentrations in the stems of 3-year-old branches sampled in July; Starch leaves Jul, NSC leaves Jul = starch and NSC concentrations (respectively) in the leaves of 3-year-old branches sampled in July; Starch pool Jul, NSC pool Jul, N pool Jul = starch, NSC and N pools (respectively) in 3-year-old branches sampled in July

Growth variables: ASG_{spring} = absolute spring biomass growth per 3-year-old branch, RSG_{spring} = relative spring biomass growth expressed as a percentage of total branch biomass in April

radial growth in the main stems of *Q. faginea* trees (Table 3). Similarly, we observed a significant negative correlation between N concentrations in the new shoots and branch NSC pools at the end of the shoot growth season (July) and the radial growth of *Q. ilex* (Table 4).

Discussion

Our results highlight the relevance of plant phenology in understanding the seasonal changes in nutrient and carbohydrate concentrations of plants, providing crucial information for the study of Mediterranean evergreen and deciduous oaks. Contrary to previous studies comparing angiosperm deciduous and evergreen conifers, they point to similar amplitude in the seasonal variability of C stores of Mediterranean oaks with contrasting leaf habit and highlight a prominent role of young branches in the winter C and N storage of evergreen oaks.

Tree phenology helps to explain branch seasonal patterns of nutrients and carbohydrates

In accordance to our first hypothesis, branch seasonal patterns of nutrients and carbohydrates were largely associated with the aboveground phenology and the patterns of leaf senescence of study species. Nutrient dynamics in the branches of both species were generally related to the growth of new shoots in spring and, in the case of K concentrations, likely also to the physiological adjustment of trees to summer drought (Milla et al. 2005). Nutrient concentrations were the highest in young expanding leaves and stems, and became subsequently diluted or retranslocated as organs matured. The less marked decrease in N observed in young leaves of both species in 2007 might be due to the fact that slightly less tender branches were collected on that second year. N and P concentrations increased also in older cohorts of stems and leaves prior to bud burst (Online Resource 1, Figs. S2 and S3), indicating the sequential allocation of nutrients from other parts of the plant to old leaves and

Table 4 Correlations between growth and storage variables in 3-year-old branches of *Quercus ilex*

Storage variables	Growth variables			
	ASG$_{spring}$ (mg branch^{-1})	RSG$_{spring}$ (% total May. branch BM)	Annual radial growth (mm)	Annual basal area increment (mm^2)
N leaves Feb (%)	0.654 (0.040)			
Starch pool Feb (mg branch^{-1})	0.952 ($<$0.001)	0.825 (0.006)		
NSC pool Feb (mg branch^{-1})	0.962 ($<$0.001)	0.826 (0.006)		
N pool Feb (mg branch^{-1})	0.952 ($<$0.001)	0.795 (0.010)		
Starch stems May (%)		–0.648 (0.031)		
NSC stems May (%)		–0.738 (0.009)		
ΔNSC$_{pool\ sources}$ (mg branch^{-1})		0.626 (0.039)		
ΔN$_{pool\ sources}$ (mg branch^{-1})		0.623 (0.041)		
N new stems Jul (%)				–0.638 (0.047)
Starch pool Jul (mg branch^{-1})			–0.673 (0.033)	–0.753 (0.012)
NSC pool Jul (mg branch^{-1})				–0.693 (0.026)

Only storage variables with significant correlations are shown. Pearson correlation coefficients along with *P* values (in parentheses) are shown only for significant correlations ($P < 0.05$). $N = 11$ trees

Storage variables: N leaves Feb = leaf N concentrations in February; Starch pool Feb, NSC pool Feb, N pool Feb = starch, NSC and N pools (respectively) in 3-year-old branches sampled in February; Starch stems May, NSC stems May = stem starch and NSC concentrations in May; ΔNSC$_{pool\ sources}$, ΔN$_{pool\ sources}$ = Difference in the NSC and N pools (respectively) in the old leaves and stems of 3-year-old branches sampled in February and May; N new stems Jul = N concentrations in the new stems of branches sampled in July; Starch pool Jul, NSC pool Jul = starch and NSC pools (respectively) in 3-year-old branches sampled in July

Growth variables: ASG$_{spring}$ = absolute spring biomass growth per 3-year-old branch, RSG$_{spring}$ = relative spring biomass growth expressed as a percentage of total branch biomass in May

stems and then to new shoots. Both N and P are crucial for protein synthesis (Garten 1976), which is enhanced during growth periods. Concentrations of K also increased in all cohorts of leaves and stems following bud burst, remaining high during summer. Similarly high K concentrations were observed in previous studies on Mediterranean evergreens during summer (Milla et al. 2005). Indeed, K plays a prominent role as osmolyte in cell turgor maintenance both during cell elongation (Boyer 1988) and during periods of drought (Ahmad et al. 2016).

The remobilization of nutrients during leaf senescence may explain the increase in P and N concentrations of the branches of *Q. faginea* during autumn, prior to leaf senescence in November–December (Montserrat-Martí et al. 2009). This does not explain the observed increase in N concentrations in the evergreen *Q. ilex*, since the leaf senescence of this species takes place throughout summer (Milla et al. 2005; Montserrat-Martí et al. 2009). Autumn is an important period for nutrient mineralization in Mediterranean-type ecosystems (Marion 1982). Consequently, external N uptake could explain the observed increase in N concentrations of *Q. ilex* during autumn, as reported for other Mediterranean evergreen oaks (Oliveira et al. 1996).

Differently to nutrient dynamics, branch seasonal patterns of starch and NSC markedly differed between study species, which can partly be explained by their contrasting leaf phenology and the differential role of their branches as C storage organs. Both starch and NSC concentrations peaked in early autumn in the branches of the deciduous species, well before the onset of leaf senescence. This is probably a result of increased C fixation in the leaves of this species after the release from summer drought. Indeed, leaves of *Q. faginea* remain green at this time of the year and senesce mainly during November and December at the study site (Montserrat-Martí et al. 2009). Our results indicate that strong C fixation may occur prior to senescence in September and October, making autumn an important C gain period for Mediterranean winter-deciduous species. Alternatively, the accumulation of NSC in the leaves of *Q. faginea* in autumn may be the result of reduced C demand for growth at this time of the year. These carbohydrates may be rapidly exported out of the branch to other storage sites (Kozlowski 1992), as starch and NSC concentrations decreased sharply during winter, matching the leaf senescence of this species. The faster decrease in starch than in NSC concentrations indicates a transient conversion of starch into SS (Fig. 2), either for cold hardening purposes (Larcher and Thomaser-Thin 1988) or for translocation to other parts of the tree (Kozlowski 1992). Starch and NSC concentrations subsequently decreased during winter in the branches of the deciduous species. As a result, *Q. faginea* displayed low branch carbohydrate concentrations prior to bud burst, concentrations that were slightly decreased further during the earlier stages of shoot growth (i.e. bud swelling), reaching seasonal minimum values. Strong reductions of branch starch concentrations during bud break have been reported for several temperate deciduous species (Schädel et al. 2009; Klein et al. 2016). Further, ring-porous oak species have been reported to resume spring cambial activity slightly earlier than bud burst, with the subsequent drain on NSC stores (Takahashi et al. 2013; Kitin and Funada 2016). However, the observed decline in the C stores of the branches of *Q. faginea* was only moderate and of short duration (only during the bud swelling phase), since starch or NSC concentrations were not decreased further during bud burst and shoot elongation.

NSC seasonal dynamics of the evergreen species lagged 2 months behind those of the deciduous. *Q. ilex* accumulated NSC in the branches throughout autumn and winter, indicating a significant positive C gain at this time of the year and storage at the branch level. These C stores were subsequently invested in new shoot growth in spring, progressively decaying to close-to-minimum values following leaf senescence in early summer. Therefore, contrary to *Q. faginea*, the decrease in starch and NSC concentrations in the branches of *Q. ilex* was broad and maintained throughout the different stages of shoot growth. These results agree with previous results on evergreen broadleaved species (Körner 2003; Rosas et al. 2013), but contrast with studies on evergreen conifers, where the spring reduction in branch NSC concentrations was small and restricted to 1-year-old foliage (Schädel et al. 2009).

Interestingly, nutrients and carbohydrates showed opposite trends in successively older branch cohorts, particularly in *Q. ilex*. Starch and NSC concentrations were progressively higher in older cohorts of leaves and stems, while the opposite was true for nutrients (Online Resource 1 Figs. S2 and S3). The decrease in nutrient concentrations as organs age is indicative of nutrient limitation and can be explained by nutrient translocation to other plant organs or by the dilution by carbon compounds (Milla et al. 2006). Mediterranean ecosystems are considered nutrient poor (Delgado-Baquerizo et al. 2011), as determined by their low litter decomposition rates (Gallardo and Merino 1993). It is, thus, not surprising that plants try to withdraw N as efficiently as possible from their senescing leaves (Pugnaire and Chapin 1993). Contrastingly, the increased accumulation of starch and NSC prior to senescence (particularly in old leaves that are about to be shed) points to a profligate use of C in these species (Millard et al. 2007; Millard and Grelet 2010).

Similar seasonal variation of branch carbohydrates between species, but larger variability of N concentrations in the deciduous oak

Partly in accordance with our second hypothesis, seasonal variations of N concentrations were sharper in the winter deciduous species, which may be indicative of a higher reliance on N remobilization and recycling from young branches in this species (Millard and Grelet 2010). These differences are likely due to the shorter life span of the leaves of *Q. faginea* and the subsequent need to recycle N as efficiently as possible (Escudero et al. 1992b), provided the limiting nature of N in Mediterranean ecosystems (Delgado-Baquerizo et al. 2011). Alternatively, the assessment of the extent of nutrient remobilization by the seasonal amplitude may underestimate the ability of *Q. ilex* leaves to remobilize nutrients throughout their lifespan, given that these leaves have longer life spans (often 3 years) than the duration of this study (2 years).

Contrary to our second hypothesis and to the long-held view that deciduous species rely more on carbohydrate stores for spring growth than evergreens, both species showed similar amplitude in the variation of the seasonal concentrations of starch and NSC of their branches. This indicates that, despite their different leaf habit, both species relied similarly on their C stores throughout the year (Hoch et al. 2003) at the branch level. Our study did not account for potential changes on stores accumulated in other parts of the tree such as roots or trunks, which may also be highly relevant for C storage in trees (Kozlowski 1992). We, thus, cannot rule out the possibility that both species remobilized resources also from other parts of the tree. Nevertheless, regarding branches, the reliance of both species on C stores seems comparable in magnitude.

Branches serve as important nutrient and carbohydrate storage sites prior to bud burst in the evergreen oak

Contrary to the prevailing view that trees do not have preferential storage sites for carbohydrates (Kozlowski 1992; Millard and Grelet 2010; Hoch 2015), our results clearly demonstrate a prominent role of old leaves and twigs in the storage and remobilization of both N and C compounds prior to bud burst in *Q. ilex*. Similar results were obtained by Cherbuy et al. (2001), who detected a significant remobilization of NSC from the old fractions of 3-year-old branches of this species. Also, previous studies on seedlings of Mediterranean evergreens (including oaks) reported a high relevance of carbohydrates stored in previous-year leaves and stems to support new shoot growth in spring (Cerasoli et al. 2004; Uscola et al. 2015). In our study, branches of *Q. ilex* that had larger N and carbohydrate stores at the beginning of the growing season and that remobilized more stores to supply the earlier stages of shoot growth, grew more in spring. While our calculations for C supplies relate only to stored C and not newly fixed carbohydrates, transfer of photosynthates from old leaves to growing shoots may also be relevant in evergreen species (Hansen and Beck 1994; von Felten et al. 2007; Uscola et al. 2015). Indeed, we observed a positive correlation between N concentrations in old leaves prior to bud burst and spring growth in *Q. ilex* (Table 4). Since the carboxylating enzyme Rubisco is also a relevant N storage protein (Ourry et al. 2001; Millard et al. 2007), our results may also indicate a significant role of old leaves as suppliers of newly fixed C to growing shoots. Nevertheless, the allocation of newly fixed C did not overrun the relationship between stored C pools and growth, which points to a prominent role of branch C stores for the spring growth of this species.

Contrary to our third hypothesis, the major storage role of branches of the evergreen was not shared by the winter deciduous oak. In *Q. faginea*, the variation in NSC and starch was largely uncoupled from shoot growth and no significant relationship could be verified between C storage pools prior to bud burst and shoot growth (Table 4). In this species, branches served as temporary storage sites for carbohydrates accumulated during summer and autumn (Fig. 2), which were probably exported to other storage sites during winter, being hence largely unrelated to spring growth. Although such translocation to other plant parts could not be assessed with our methodological approach, studies performed under more humid conditions reported NSC increments during autumn and winter in the main stem of deciduous oaks (El Zein et al. 2011; Pérez-de-Lis et al. 2017), suggesting that NSC are partially allocated from branches to storage compartments at the end of the growing season. Further, previous studies indicate that winter deciduous trees from temperate regions store nutrients preferentially in the roots and trunks (Chapin et al. 1990; Millard 1996; Millard and Grelet 2010), including temperate oaks like *Q. petraea* and *Q. pyrenaica* (Barbaroux et al. 2003; Salomón et al. 2016). Finally, branches of winter deciduous species rapidly become autotrophic (Keel and Schädel 2010; Landhäusser 2011). In our study, branches of the winter deciduous that grew more in spring (and hence had more foliage) were also able to accumulate more carbohydrate and N stores at the end of the growing season.

Conclusions

Both oak species showed similar amplitude in the seasonal variation of their branch C and nutrient stores and also comparable seasonal dynamics of nutrient (N, P and K) concentrations in their branches. However, seasonal dynamics of C storage compounds (starch and NSC) were different in the two species. In the evergreen, branch storage and remobilization was crucial for new shoot growth in spring. Accordingly, positive correlations were observed between branch N and C storage pools and spring growth, and carbohydrate concentrations peaked before bud burst in all branch organs. Contrastingly, branches of the winter deciduous showed only a transient role as sites for C storage. These branches accumulated C stores during summer and early autumn, which were subsequently consumed locally or retranslocated to other parts of the tree (presumably the trunk and roots) during winter, leading to seasonal minimum concentrations. Although the present study focused on branches only, without accounting for other parts of the tree like the roots or the trunk with potential implications on C storage, our results challenge previous assumptions on the lower relevance of branch C storage for the spring growth of evergreen trees and call for further studies where the nutrient and C use of closely related pairs of coexisting evergreen and deciduous trees is compared.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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