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Seasonal variability of dry matter content and its relationship to shoot growth and non-structural carbohydrates

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Summary

- This study assesses how different phases of shoot growth underlie seasonal change in leaf and stem dry matter content (LDMC and SDMC) of 12 woody Mediterranean species. We also explore the relationship of LDMC with non-structural carbohydrate (NSC) concentrations and compare the seasonal *vs.* interspecies variability of LDMC.
- LDMC, SDMC and shoot elongation rate (SER) were measured on a monthly basis for a minimum of 12 months. Bud growth rate (BGR) and NSC concentrations were also assessed in several of the study species.
- LDMC and SDMC decreased during shoot elongation in spring and increased in summer, showing a significant negative correlation with SER, but were unrelated to BGR. Half of the species analysed showed a positive relationship between LDMC and NSC.
- Seasonal fluctuations of LDMC within species were higher than interspecies differences, and species ranking was significantly affected by the month of sampling, except during winter months.
- Seasonal changes in LDMC and SDMC are mainly related to shoot elongation phenology and NSC sink-source relationships between old and growing organs can explain this relationship in some species. Due to the high seasonal variability in LDMC, we recommend collecting samples for comparative purposes as close to the winter as possible.

Keywords: Leaf dry matter content, LDMC, Mediterranean, shoot growth, phenology, leaf water status, functional classifications, leaf traits.

Introduction

Over the last decades great efforts have been made to identify key traits suitable to simplify the huge taxonomical diversity of plants into a series of ecologically relevant functional types (Grime *et al.*, 1997; Westoby, 1998; Weiher *et al.*, 1999; Cornelissen *et al.*, 2003; Wright *et al.*, 2004). Among such traits, the leaf dry matter content (LDMC), the ratio of leaf dry mass to saturated fresh mass, has gained recognition as a consistent and easy-to-measure trait, suitable for large screening programmes (Wilson *et al.*, 1999; Garnier *et al.*, 2001a; Garnier *et al.*, 2004). At the whole-plant level, LDMC correlates negatively with potential relative growth rate (Cornelissen *et al.*, 2003 and references therein) and with potential decomposability of plant tissues (Kazakou *et al.*, 2006). Thus LDMC can be a measure of the trade-off between rapid production of biomass and efficient nutrient conservation (Grime *et al.*, 1997; Wilson *et al.*, 1999; Weiher *et al.*, 1999).

Several studies show that LDMC can vary markedly during the year, with a minimum in spring and a maximum in winter or summer (Ritchie & Shula, 1984; Devi *et al.*, 1996; Tognetti *et al.*, 2000). Putative factors explaining the seasonal variability of LDMC include organ growth phenology (Teskey *et al.*, 1984; Davis & Mooney, 1986; Gross & Koch, 1991; Montserrat-Martí *et al.*, 2004; Palacio & Montserrat-Martí, 2005), and osmotic adjustments in response to winter cold and summer drought (Doi *et al.*, 1986; Gross & Koch, 1991; Ögren, 1999). LDMC relates to tissue anatomy, being indicative of the proportion of light (i.e. mesophyll/parenchyma and epidermis) versus dense (i.e. sclerenchyma and vascular tissues) tissues (Garnier and Laurent, 1994). It also relates to leaf chemistry, being affected by the concentration of non-structural compounds (mainly sugars and low molecular weight proteins) in plant cells (Ögren, 1999;

Jongebloed *et al.*, 2004). Both leaf anatomy and chemistry change during shoot growth, while leaf chemistry is also affected by leaf productivity, cold hardening and senescence. Nevertheless, the underlying physiological factors responsible for the seasonal variation in LDMC remain mostly unexplored. Here we investigate the possible underlying role of non-structural carbohydrates, which account for an important fraction of the carbon (C) mobile pools in plants (Körner, 2003), are related to C source/sink dynamics and have important osmotic properties (Ögren, 1999).

The seasonal variability of LDMC may compromise the classifications obtained with it (Garnier *et al.*, 2001a). Assessing the relative extent of the inter-specific *vs.* the seasonal variability is, therefore, important for interpreting LDMC and related parameters in functional studies. From the limited number of studies that have attempted to assess the importance of the seasonal variability of LDMC we know that its seasonal variability may be higher than its inter-annual or spatial variability, and this may affect species ranking based in LDMC in some cases (Garnier *et al.*, 2001a). However, a recent study on the seasonal dynamics of LDMC in 30 Mediterranean species found no significant differences due to the season, although spring was not considered in the analysis (Saura-Mas & Lloret, 2007). Here, we measure the seasonal variation of LDMC on a more detailed, monthly, basis. We expand the analysis to stems (SDMC) and further try to assess if the different phases of shoot growth, i.e. organogenesis and expansion, and the seasonal changes in NSC concentrations (soluble sugars and starch) underlie the extent of seasonal variation in dry matter content. Specifically, the objectives of this study were to: 1) assess if the phenology of shoot extension and organogenesis and the changes in NSC concentrations can explain the seasonal variation of LDMC and SDMC; 2) compare the seasonal *vs.* the inter-specific variability of LDMC and SDMC;

and 3) evaluate the stability of species ranking based on dry matter content across seasons and shoot organs. We hypothesized that LDMC and SDMC will be correlated to the shoot elongation rate and NSC concentrations; and that within species variation in LDMC along the year should be low as compared to inter-specific variability. Our current analysis should help to pinpoint the most suitable periods of the year, phenological stages and shoot organs to obtain dry matter content measures readily comparable among species.

Materials and Methods

Study species, period and sites

Twelve woody species of various growth forms and leaf habits native to Mediterranean shrublands and woodlands from the Iberian Peninsula were selected for analysis (Table 1). One population of each species was studied for a minimum of 12 months between 1999 and 2006 (Table 1). Sampling was conducted on a monthly basis. Populations were located between the middle Ebro Valley and the Pre-Pyrenees (NE Spain) in an area of approximately 100 km^2 and an altitudinal range from 320 to 1380 m a.s.l. (Table 1). For more details about the study area see Guerrero Campo (1998) and Palacio *et al.* (2006).

Dry matter content measurements

In the two tree species, *Q. ilex* subsp. *ballota* and *Q. faginea*, one branch older than three-year was collected from the same ten marked individuals every month. In the remaining species, branches were randomly collected from ten different individuals within the study population at every sampling date. Once in the laboratory, samples were processed following the standard methodology described in Garnier *et al.* (2001b)

and Cornelissen *et al.* (2003). Branches were set at full hydration by cutting under water the three most proximal centimetres of the stem of each branch and immersing the first 3–4 cm of the stem in distilled water. Hydrating branches were covered by a wet plastic bag and kept at 4 ºC for 24 h. Full hydration weights were obtained for samples of leaves and stems in each hydrated branch. When present, current-year, one- and two-year-old cohorts were measured separately. Most sub-shrubs studied are seasonally dimorphic and, consequently, display two different types of leaves throughout the year; leaves from short and leaves from long branches (Orshan, 1989). Most of the year, plants bear leaves on short branches, whereas leaves from long branches occur mainly in spring and summer (Palacio *et al.*, 2006). For this reason, we measured the leaves of short branches only. However, leaves from short branches were too small to be measured separately, and hence whole short branches (with leaf biomass accounting for more than 95% of their total biomass) were collected instead of individual leaves. Subsequently, samples were oven-dried at 60 ºC to a constant weight and dry weights were obtained. All weighing was conducted to the nearest 0.01 mg (MC1, Sartorius AG, Goettingen, Germany). Dry matter content (DMC, mg g^{-1}) of leaves (LDMC) and stems (SDMC) were calculated as:

$$DMC = M_d / M_f \quad (1)$$

where M_d (mg) was the dry weight and M_f (g) the weight at full hydration of a given sample.

Shoot elongation and organogenesis

Shoot elongation was measured differently in sub-shrubs than in trees or shrubs. In the former species, destructive analyses were required because of the small size of branches

(Palacio & Montserrat-Martí, 2005, 2006). Accordingly, shoot growth was assessed on 15 marked adult individuals per species. At each sampling date, three two-year-old branches were collected from different positions within the canopy of each plant. Samples were pressed and stored in a herbarium until shoot length was measured under a stereo-microscope fitted with an ocular micrometer (40x, MS5 Leica Microsystems, Heerbrugg, Switzerland). The length of one current-year shoot per branch was measured from the insertion point on the stem to the tangent line between the apices of the most apical green leaf. Trees and shrubs had larger branches, and shoot elongation was assessed non-destructively. In *C. laurifolius, B. fruticosum, L. implexa* and *A. ovalis,* branch demography was monitored in ten marked individuals per species. One well-developed two-year-old branch was randomly selected at the mid-crown of each marked individual. Drawings were obtained for each branch every month, showing the numbers of green, senescent and dry leaves and the length of current-year shoots, as described by Milla *et al.* (2004). For both sub-shrubs and the above four shrub species, shoot elongation rate (SER, mm day^{-1}) of marked plants was calculated at every sampling date by:

$$SER = (L_n - L_{(n-1)}) / T \qquad (2)$$

Where L_n (mm) was the mean shoot length of each marked individual on month n, $L_{(n-1)}$ (mm) the mean shoot length of each marked individual on the previous month (n-1), and T (days) the period elapsed between months (n-1) and n.

In the two tree species analyzed, *Q. faginea* and *Q. ilex* subsp. *ballota*, shoot elongation dynamics were assessed by visual estimations of the phenology of shoot growth in the canopy of 15 marked individuals. Previous studies on these species indicated that isolated branches were not representative of the growth dynamics at the whole-canopy

level. Indeed, the variability found between sun and shade branches and between branches from upper and lower parts of the canopy of a same individual was sometimes higher than the variability between individuals (G. Montserrat-Martí, unpublished results). To overcome these limitations, we identified 13 easy-to-recognize phenophases that summarized the annual phenological cycle of both species and estimated the percentage of branches in the canopy of 15 marked trees showing each of these phenophases on a monthly basis. Observations on the occurrence of the different phenophases related to shoot elongation growth were then combined to assess shoot growth dynamics of both species.

Shoot organogenesis, i.e. bud development, was assessed in sub-shrubs as described by Palacio and Montserrat-Martí (2005, 2006). Ten, two-year old branches were collected randomly from ten different individuals on a monthly basis. Buds were examined under a stereo-microscope fitted with an ocular micrometer (10x and 40x, MS5 Leica Microsystems, Heerbrugg, Switzerland). The total number of leaf primordia at each sampling date (hereafter N) was counted in one bud per branch. The bud growth rate (BGR, No. primordia day^{-1}) of each species at every sampling date was calculated using the following formula:

$$BGR = (N_n - N_{(n-1)}) / T \quad (3)$$

Where N_n was the mean number of leaf primordia contained in the buds in month n, $N_{(n-1)}$ was the mean number of leaf primordia contained in the buds in the previous month (n-1) and T (days) the period elapsed between months (n-1) and n.

Non-structural carbohydrate concentrations

Soluble sugars (SS), starch and total non-structural carbohydrate (NSC) concentrations were measured on the leaves of eight of the 12 study species. These included all sub-shrubs and the two trees. In sub-shrubs, leaf samples were collected from five randomly selected adult individuals, whereas in trees leaf samples were collected from the same five marked individuals on every sampling date. Samples were stored at -20˚ C until freeze-dried (Cryodos, Telstar Industrial SL, Terrasa, Spain) and milled to a fine powder (IKA MF10, IKA-Werke, Staufen, Denmark). Soluble sugars were extracted with 80% (v/v) ethanol and concentrations were determined colorimetrically using the phenol-sulphuric method of Dubois *et al*. (1956) as modified by Buysse & Merckx (1993). Starch and complex sugars remaining in the undissolved pellet after ethanol extractions were enzymatically reduced to glucose and analyzed as described in Palacio *et al*. (2007a). Non-structural carbohydrates measured after ethanol extraction are referred to as soluble sugars (SS), carbohydrates measured after enzymatic digestion in glucose equivalents are referred to as starch and the sum of SS and starch measured in glucose equivalents are referred to as total non-structural carbohydrates (NSC).

Statistical analyses

The distribution of SER data could not be transformed to follow a normal distribution. Therefore, the relationship between LDMC, SDMC and shoot elongation and organogenesis was explored by calculating Spearman correlation coefficients. In every species, mean monthly LDMC and SDMC data were correlated, separately for each organ, with the mean SER (or the mean percentage of branches undergoing shoot elongation in the case of *Q. faginea* and *Q. ilex* subsp. *ballota*) of that same month. In sub-shrubs, LDMC and SDMC data were also correlated with values of BGR of the same month to assess the relationship with shoot organogenesis. In those species for

which NSC data were available (all sub-shrubs and the two trees), these were correlated with values of LDMC for the same dates. Data were normally distributed and hence the relationship between both variables was assessed by Pearson correlation tests. Correlation analyses were conducted by using SPSS 14.0 (SPSS Inc., Chicago, IL, USA).

In view of the large differences in the leaf habit of study species, the comparison between the seasonal and the inter-specific variability of the dry matter content was conducted using residual maximum likelihood (REML) (Genstat 9th ed, VSN International Ltd., UK). All species but the two trees (*Q. faginea* and *Q. ilex* subsp. *ballota*) were analysed together, with: (a) organ (i.e. stems or leaves), month and species as fixed factors, and (b) site and year of study as random factors. A separate analysis of the LDMC data of the four shrub species (for which two year records were available) showed that "year" as a fixed factor explained only 0.16% of the total variance. Data from the two trees were not considered because they come from repeated measures. All interactions between fixed factors were included in the model. The percentage of the total variance explained by each fixed factor was calculated from the residual variance estimates, by sequentially adding fixed terms to the model.

To specifically account for the effect of the seasonal variability of LDMC on species ranking, we calculated the Spearman rank correlation coefficients of the ranking of species obtained with the average LDMC of different months. Only those months when leaves were mature were included in the analysis to avoid the effect of changes in LDMC due to leaf development. Species ranking of different months were tested against each other. In addition, a "random" species ranking was obtained by combining

LDMC data from randomly selected months different among species. This was intended to mimic the effect of combining LDMC data of species sampled at different times of the year.

Results

Relationship between LDMC, SDMC, shoot growth and NSC concentrations

For most species LDMC and SDMC were minimum when shoot elongation rate was at its highest (Figs. 1 and 2). The greatest seasonal changes in LDMC and SDMC occurred during the period of shoot elongation in spring and early summer, while dynamics tended to stabilize by August or September and remained without much variation throughout autumn and winter (Figs. 1 and 2). Indeed, differences between spring minima and late summer maxima were significant for most species and organs ($P < 0.01$, results not shown), except for the old leaves of *Q. ilex* subsp. *ballota* ($P > 0.05$). Accordingly, LDMC and SDMC were negatively correlated to SER in many of the study species, whereas they were uncorrelated to BGR (Table 2). In *L. subulatum*, leaves showed high values of LDMC during late summer (Fig. 1) and hence LDMC and SDMC were only weakly correlated to SER (Table 2). Such low summer values might be due to the difficulty of separating the dry leaves of this species prior to weighing. It is remarkable that LDMC and SDMC underwent similar seasonal trends in growing (current-year) and non-growing (old) cohorts of all species (Figs. 1 and 2), except for the old leaves of *Q. ilex* subsp. *ballota* which showed no seasonal change.

The relationship between LDMC and NSC concentrations was strong and significant for many of the species analysed, however, trends varied between species (Fig. 3). LDMC showed a positive correlation with NSC concentrations in half of the species considered

in the analysis (Fig. 3). On the contrary, LDMC correlated negatively with the NSC concentrations of the leaves of *E. horridum*, and the relationship was not significant for *L. subulatum, L. suffruticosum* and *O. fruticosa* (Fig. 3). In most species NSC accounted for an important fraction of the total leaf dry mass, reaching up to 50 % of LDMC (Fig. 3).

Seasonal vs inter-specific variability of LDMC and SDMC

The analysis of the variance components of the dry matter content showed that all of the fixed factors included in the model had a significant effect, as well as their interactions (Table 3). Of the three fixed factors considered, "month" explained most of the variability, whereas the percentage of the total variance explained by "organ" and "species" was comparatively low (Table 3). The interaction between "month" and "species" was significant and explained 20% of the total variance in the dry matter content. This indicates that the comparison between species was affected by the month of sampling. Indeed, the analysis of the stability of species ranking among months by Spearman rank correlation tests showed that species ranking based on LDMC changed significantly from month to month (Table 4). Most correlations yielded not significant coefficients, and only during winter months (January, February and December), were classifications stable (i.e. correlation coefficients were significant).

Discussion

Relationship between dry matter content, shoot growth and NSC concentrations

Shoot growth is the result of two processes: the differentiation of organ primordia from meristems, i.e. organogenesis, and the extension of these primordia into fully developed

organs (Champagnat *et al.*, 1986). Our results indicate that the negative relationship between LDMC, SDMC and shoot elongation in spring is widespread among Mediterranean woody species, whereas such relationship does not hold for shoot organogenesis. An increase in the saturated weight / dry weight ratio of leaves (the inverse of LDMC) during spring growth has been reported for several mediterranean woody species before (Davis & Mooney, 1986; Tognetti *et al.*, 2000). Such spring changes were attributed to ontogenetic processes related to the onset of shoot elongation (Davis & Mooney, 1986). One of the prerequisites for bud burst is increased bud and shoot hydration (Bradford & Hsiao, 1982; De Faÿ *et al.*, 2000). Expanding cells adjust their osmotic potential and cell wall elasticity to maintain adequate turgor pressure throughout the growth process (Boyer, 1988; Cosgrove, 1993; Van Volkenburgh, 1999). Consequently, the mechanisms used by cells to maintain this pressure may increase the capacity of organs to gain water when set at full turgidity, hence leading to reduced LDMC and SDMC during shoot elongation. Once cell expansion has ceased, dry matter accumulates in cells leading to the observed increase in LDMC during summer. Part of such dry matter may come in the form of NSC, which accounted for up to 50 % of LDMC. Also, NSC concentration was found to be positively correlated to LDMC in half of the species analysed. The accumulation of NSC in new leaves could be the result of a progressive increase in their net photosynthetic rate, once respiratory demands associated to growth decrease and leaves become net sources of C. Indeed, despite the severity of summer drought in Mediterranean climate, several studies have reported positive net photosynthetic rates in the leaves of Mediterranean species at this time of the year (Kyparissis & Manetas, 1993; Kyparissis *et al.*, 1997), which results in an increase in the NSC pools of leaves (Körner, 2003; Palacio *et al.*, 2007a, b).

While the tight relationship between the dry matter content and shoot elongation may be explained by changes in cell wall elasticity and osmotic potential during cell expansion, our results demonstrate that also fully mature, non-growing tissues undergo similar decreases in LDMC and SDMC during spring. The spring reduction in dry matter content of mature organs was lower than in growing organs, yet it was significant for most species. Similar results have been reported before (Gross & Koch, 1991; Borchert, 1994; Palacio & Montserrat-Martí, 2005; Milla *et al.*, 2007; but see Teskey *et al.*, 1983). The decrease of SDMC in old stems can be explained by an increase in the water flow through xylem vessels to supply elongating organs. However, old leaves are more isolated from the conductive system than stems, and other physiological mechanisms could be involved. Our results suggest that changes in NSC concentrations caused by C transfers from old (sources) to growing (sinks) organs could be related to the spring decrease in LDMC of old leaves. NSC concentrations decreased in old leaves concurrently with the decrease in LDMC during shoot elongation in most of the species included in our analysis that bore old leaves in spring. These results could be indicative of a translocation of mobile carbohydrates from old leaves, with a positive net photosynthetic rate, to new expanding leaves, which are still not fully autotrophic. Some of the above species (like *S. montana* and *S. lavandulifolia*) are leaf exchangers, i.e. they exchange leaf cohorts in spring, and hence the decrease in NSC was probably related to a recycling of resources associated to leaf senescence (Palacio *et al.*, 2007b). Yet in other species, such as *Q. ilex* subsp. *ballota,* leaf senescence was not involved, as leaves can live up to four years and leaf senescence does not overlap with shoot elongation (Escudero *et al.*, 1992). Also, a previous study on the leaf exchanger *C. laurifolius* found that, although the withdrawal of NSC could explain the decrease in LDMC during the earlier stages of leaf senescence in spring, subsequent changes in

osmolyte (mostly SS) content were unrelated to leaf water content (the reverse of LDMC) during late senescence (Milla *et al.* 2007). Non-structural carbohydrate concentrations were also unrelated to LDMC in some of the species analysed in this study, yet in others, such as the cushion plant *E. horridum*, the relationship was negative. Although differences in leaf phenology among species could partly account for these contrasting results, further research on the role of NSC and other possible underlying mechanisms behind LDMC seasonality is needed.

Seasonal vs. inter-specific variability of LDMC

The within-species variability of LDMC during the year was greater than inter-specific variability. This contrasts with previous studies where seasonal variability of LDMC was found to be low, though such studies were based on seasonal instead of monthly data, and not all seasons were covered (Al Haj Khaled *et al.*, 2005; Saura-Mas & Lloret, 2007, but see Garnier *et al.*, 2001a). Our results showed that species ranking is affected by the month of sampling. This means that the high seasonal variability of LDMC can affect the consistency of functional classifications based on this trait. Garnier *et al.* (2001a) showed that inter-season variation affects species ranking based on traits such as leaf nitrogen concentration or LDMC. They attributed the observed seasonal effect to the lower LDMC in spring and to the fact that some of the sampled leaves may still be immature. These observations agree with our results, as spring was found to be the period when LDMC was more variable. Nevertheless, we have also shown that LDMC of old leaves, which are fully mature, tends to fluctuate in spring. These results highlight the need of avoiding spring collection of samples for measuring LDMC for comparative purposes, even if leaf expansion has ceased. Garnier *et al.* (2001a) suggested July as the optimum period for collecting leaves for functional classification

purposes, at least for woody species growing in a Mediterranean climate in the Northern hemisphere. Here we recommend delaying sampling of Mediterranean species to winter, the only time of the year when species ranking based on LDMC was found to be stable (see Table 4). This recommendation excludes winter deciduous species, which we recommend sampling as close to the winter as possible but when leaves are still not senescent. These sampling criteria may be adapted to woody species growing in other areas different to the Mediterranean by considering the time of the year when shoot elongation is completely finished and species are close to dormancy.

Conclusions

We have shown that seasonal changes in LDMC and SDMC are mainly related to shoot elongation phenology, spring being the period of the year when LDMC and SDMC undergo the greatest oscillations and reach minimum values. Results are similar for all cohorts analyzed, irrespective of their degree of maturation. Seasonal changes in LDMC could be explained by changes in the concentrations of NSC associated to C source / sink relationship between organs in some species, yet not all species conformed to this pattern. The large seasonal variability in LDMC can hinder the detection of interspecies differences and affect the classifications based on it, except during winter months, when species ranking remained stable. Thus, we recommend collecting samples for comparative purposes based on LDMC in winter, or as close to the winter as possible, even when mature leaves or stems are to be collected.

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Figure legends

Figure 1. Seasonal trends of shoot elongation rate (SER, mm day^{-1}) (—) and dry matter content (DMC, mg g^{-1}) in the current-year leaves (△) and stems (○), and one-year old leaves (▲) and stems (●) of the six species of sub-shrubs analyzed. Values are means ± SE. n= 10 for DMC and n=15 for SER.

Figure 2. Seasonal trends of shoot elongation rate (SER, mm day^{-1}) and dry matter content (DMC, mg g^{-1}) in the current-year leaves (△) and stems (○), one-year-old leaves (▲) and stems (●), and two-year-old leaves (△) and stems (○) of the shrubs and trees analyzed. For both *Q. faginea* and *Q. ilex* subsp. *ballota* SER is expressed as a percentage (%).Values are means ± SE. n= 10 for DMC and n=10 for SER.

Figure 3. Relationship between the leaf dry matter content (LDMC) and the total non-structural carbohydrate (NSC) concentrations of some of the study species. Pearson correlation coefficients (r) and P-values (P) are shown, except in *E. horridum* where the Spearman correlation coefficient is shown.

Table 1. Main characteristics of study species and sampling sites.

Species	Growth form	Leaf habit	Period of study	Location[a]	Altitude (m a.s.l.)	P (mm)	T (ºC)	Study site (UTM)
Amelanchier ovalis Medicus	Shrub	Winter deciduous	1999-2000	Luesia (Hu)	780	742	11.9	30T XM6397
Bupleurum fruticosum L.	Shrub	Evergreen	1999-2000	Orés (Hu)	760	633	12.3	30T XM6682
Cistus laurifolius L.	Shrub	Evergreen	1999-2000	Luesia (Hu)	780	742	11.9	30T XM6397
Echinospartum horridum (Vahl) Rothm.	Sub-shrub	Winter deciduous with photosynthetic stems	2002-2003	Las Peñas de Riglos (Hu)	1380	1247 (*)	8.0 (*)	30T XN8908
Lepidium subulatum L.	Sub-shrub	Seasonally dimorphic	2002-2003	Villamayor 2 (Z)	320	403	14.1	30T XM8820
Linum suffruticosum L.	Sub-shrub	Seasonally dimorphic	2002-2003	Villamayor 2 (Z)	320	403	14.1	30T XM8820
Lonicera implexa Aiton	Vine	Evergreen	1999-2000	Orés (Hu)	760	633	12.3	30T XM6682
Ononis fruticosa L.	Sub-shrub	Winter deciduous	2003-2004	Bernués (Hu)	810-1020	693	12.0	30T YN0108
Quercus faginea Lam. subsp. *faginea*	Tree	Winter deciduous	2005-2006	Agüero (Hu)	750-760	635	13.4	30TXM8086
Quercus ilex L.subsp. *ballota* (Desf.) Samp.	Tree	Evergreen	2005-2006	Agüero (Hu)	750-760	635	13.4	30TXM8086
Salvia lavandulifolia Vahl	Sub-shrub	Seasonally dimorphic	2002-2003	Villamayor 1 (Z)	340	403	14.1	30T XM8920
Satureja montana L. subsp *innota* (Pau) Font Quer	Sub-shrub	Seasonally dimorphic	2003-2004	Lasieso (Hu)	675	653	12.1	30T YM1099

Precipitation (P; mm) and temperature (T; ºC) values were obtained from the closest weather station to study populations (always located less than 10 km away from study sites). No weather information was available for the population of *Echinospartum horridum* (Las Peñas de Riglos, Huesca). Therefore, mean annual rainfall and temperature values for this site (*) were extrapolated from values of the meteorological station of Jaca (31 years of record, 840 m a.s.l.) located 25 km away, following the vertical gradients proposed for the Pyrenees by López-Moreno (2005) and García-Ruíz et al. (1985), respectively.

[a] Hu = Huesca, Z = Zaragoza.

Table 2. Spearman correlation coefficients between the leaf and stem dry matter content (LDMC and SDMC, respectively) and the shoot elongation rate (SER) and bud growth rate (BGR) of study species.

Species	**SER**		**BGR**	
	Stems	**Leaves**	**Stems**	**Leaves**
Amelanchier ovalis	-0.685	-0.579	-	-
Bupleurum fruticosum	**-0.782***	**-0.629***	-	-
Cistus laurifolius	**-0.842****	**-0.661***	-	-
Echinospartum horridum	-0.399	-0.600	-0.538	-0.667
Lepidium subulatum	**-0.636***	**-0.587***	0.014	-0.343
Linum suffruticosum	-0.524	-0.315	0.189	-0.559
Lonicera implexa	-0.471	-0.236	-	-
Ononis fruticosa	**-0.619***	-0.600	-0.237	-0.800
Quercus faginea	**-0.710****	**-0.939*****	-	-
Quercus ilex	**-0.841*****	-0.297	-	-
Salvia lavandulifolia	**-0.706****	**-0.635***	-0.350	-0.036
Satureja montana	**-0.893*****	**-0.818****	0.152	0.030

Significant correlations are indicated in bold. Asterisks indicate level of significance: * $P < 0.05$, ** $P < 0.01$; *** $P < 0.001$.

Table 3. Results of REML variance components analysis for the dry matter content of the leaves and stems of all study species (but *Q. faginea* and *Q. ilex* subsp. *ballota*). Wald tests and percentage of the total variance explained by fixed effects (TVE; %) calculated by sequentially adding terms to fixed model. "Site" and "year of study" were included as random effects. P-values calculated from chi-square distribution for Wald tests. d.f. = degrees of freedom.

Fixed term	Wald statistic	d.f.	TVE (%)	P-value
Month	10532.34	11	37.6	<0.001
Organ	3199.29	1	11.5	<0.001
Species	976.76	9	3.4	<0.001
Month x Organ	1251.34	11	4.3	<0.001
Month x Species	5751.14	97	20.4	<0.001
Organ x Species	2459.14	9	8.8	<0.001
Month x Organ x Species	1759.62	69	6.5	<0.001

Table 4. Spearman correlation coefficients comparing the rankings of species according to their average LDMC data from different months. The "Random" analysis was made by ranking species LDMC data from randomly selected months different among species. Significant correlations after applying Bonferroni correction for multiple correlations are indicated in bold ($\alpha = 0.001$).

	Feb	**Mar**	**Jul**	**Aug**	**Sep**	**Oct**	**Nov**	**Dec**	**Random**
Jan	**1.000**	0.405	0.310	0.048	0.500	0.721	0.167	**1.000**	-0.048
Feb		0.405	0.310	0.048	0.500	0.721	0.167	**1.000**	0.048
Mar			-0.190	0.071	0.095	0.190	-0.762	0.405	-0.167
Jul				-0.109	0.664	-0.176	-0.539	-0.283	-0.350
Aug					0.115	-0.267	-0.333	0.067	-0.100
Sep						0.079	-0.235	0.483	-0.100
Oct							-0.067	0.733	-0.418
Nov								-0.067	0.406
Dec									-0.067

Fig. 1.

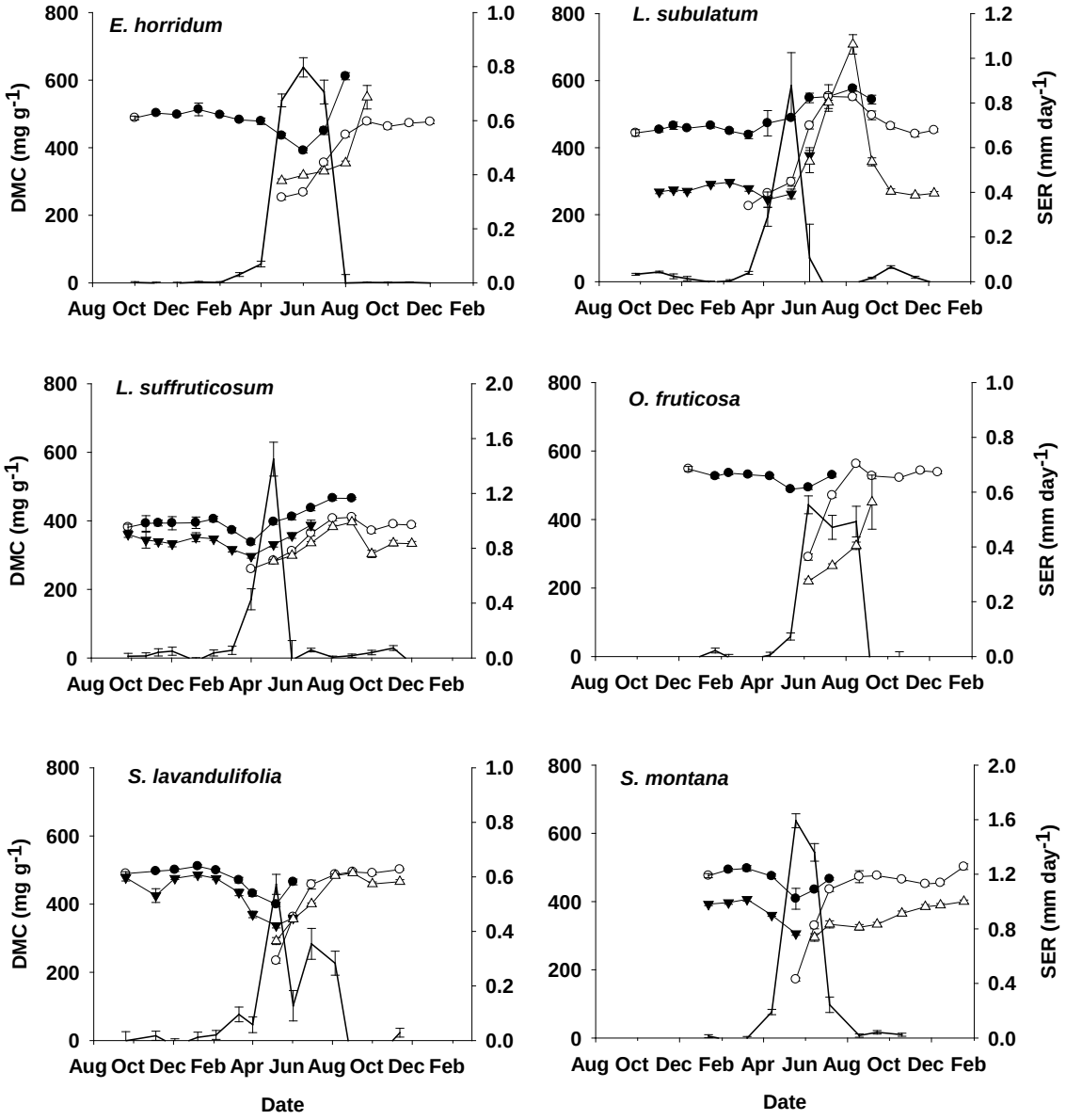

E. horridum
L. subulatum
L. suffruticosum
O. fruticosa
S. lavandulifolia
S. montana
DMC (mg g-1)
SER (mm day-1)
Aug Oct Dec Feb Apr Jun Aug Oct Dec Feb
Date

Fig. 2.

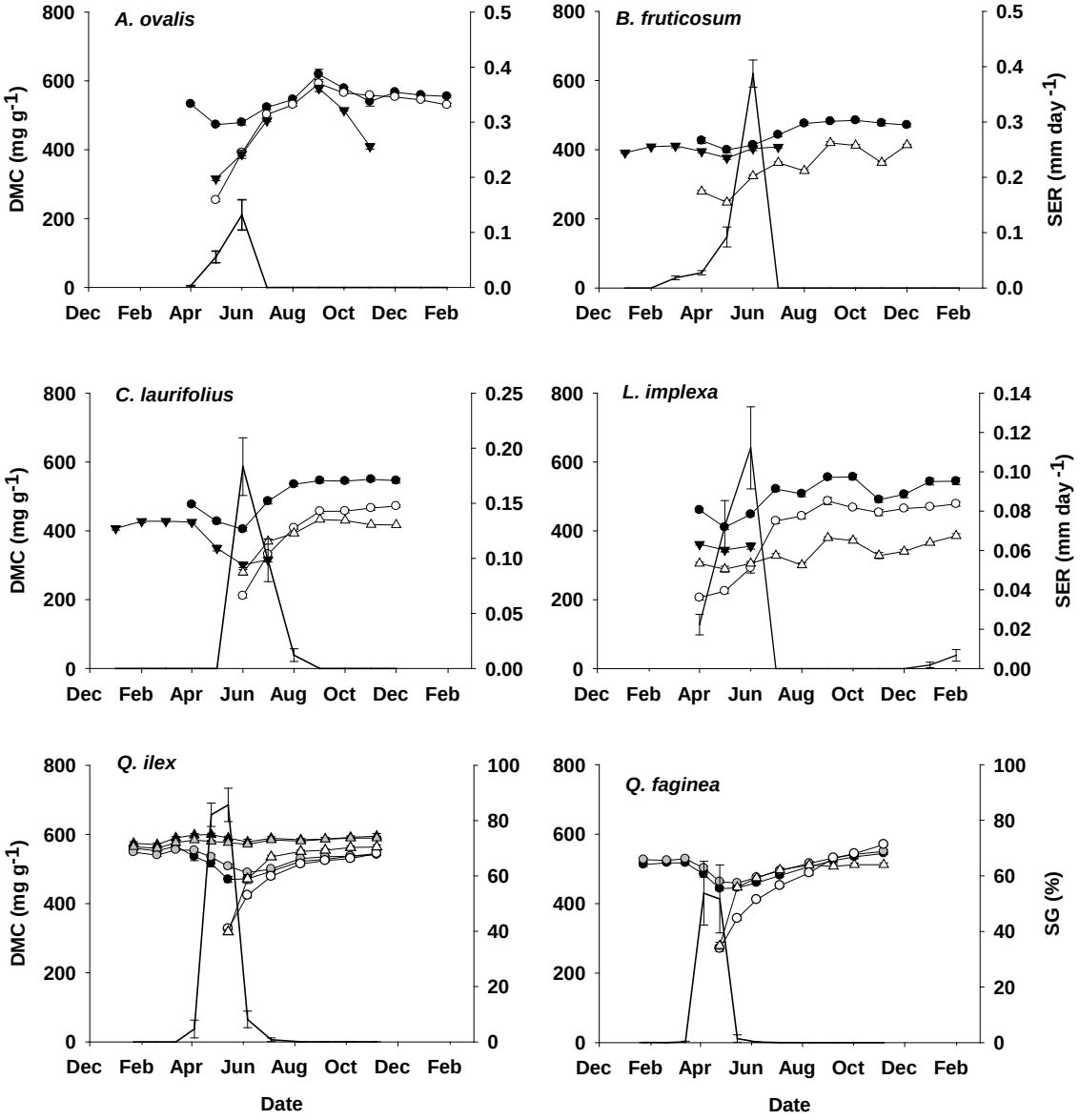
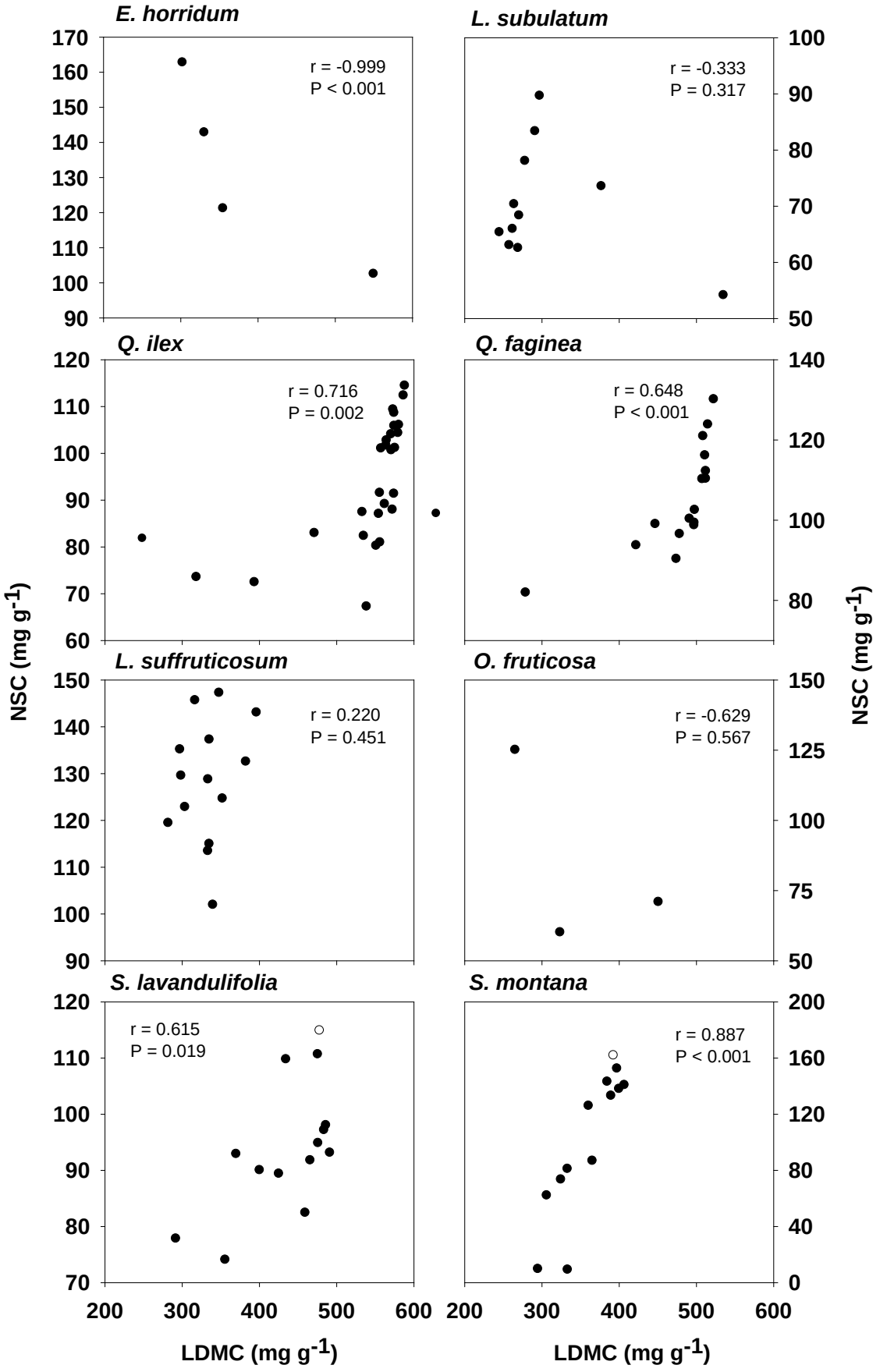

A. ovalis
B. fruticosum
C. laurifolius
L. implexa
Q. ilex
Q. faginea
DMC (mg g-1)
SER (mm day-1)
SG (%)
Dec Feb Apr Jun Aug Oct Dec Feb
Date

Fig. 3


E. horridum
r = -0.999
P < 0.001
L. subulatum
r = -0.333
P = 0.317
Q. ilex
r = 0.716
P = 0.002
Q. faginea
r = 0.648
P < 0.001
L. suffruticosum
r = 0.220
P = 0.451
O. fruticosa
r = -0.629
P = 0.567
S. lavandulifolia
r = 0.615
P = 0.019
S. montana
r = 0.887
P < 0.001
NSC (mg g-1)
LDMC (mg g-1)