Tree Species Effect on Litter Decomposition and Nutrient Release in Mediterranean Oak Forests Changes Over Time

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ABSTRACT

Tree species can affect the decomposition process through the quality of their leaf fall and through the species-specific conditions that they generate in their environment. We compared the relative importance of these effects in a two-year experiment. Litterbags containing leaf litter of the winter-deciduous *Quercus canariensis*, the evergreen *Q. suber* and mixed litter were incubated beneath distinct plant covers. We measured litter carbon loss, 9 macro- and micronutrients and 18 soil chemical, physical and biological parameters of the incubation environment.

Tree species affected decay dynamics through their litter quality and, to a lesser extent, through the induced environmental conditions. The deciduous litter showed a faster initial decomposition but left a larger fraction of slow decomposable biomass compared to the perennial litter; in contrast the deciduous environment impeded early decomposition while promoting further carbon loss in the latter decay stages. The interaction of these effects led to a negative litter-environment interaction contradicting the "home-field advantage" hypothesis. Leaf litter N, Ca and Mn as well as soil N, P and soil moisture were the best predictors for decomposition rates. Litter N and Ca exerted counteractive effects in early versus late decay stages; Mn was the best predictor for the decomposition limit value, that is, the fraction of slowly decomposable biomass at the later stage of decomposition; P and soil moisture showed a constant and positive relation with carbon loss. The deciduous oak litter had a higher initial nutrient content and released its nutrients faster and in a higher proportion than the perennial oak, significantly increasing soil fertility beneath its canopy.

Our findings provide further insights into the factors that control the early and late stages of the decomposition process and reveal potential mechanisms underlying tree species influence on litter decay rate, carbon accumulation and nutrient cycling.

Keywords: decomposition limit value, lignin, litterbag, litter chemistry, *Quercus*, soil fertility, plant-soil interactions,

INTRODUCTION

Differences between tree species litter decomposition have commonly been related to distinct substrate quality with litter C:N and N:P ratios, lignin content, Ca and Mn concentration emerging as the main rate-controlling factors (Melillo and others 1982; Cornelissen and others 2006; Hobbie and others 2006; Cornwell and others 2008; Güsewell and Gessner 2009; Berg and others 2010). But tree species can also alter decomposition rates indirectly through effects on environmental conditions. For example, tree species can induce changes in soil fertility, microclimate and faunal and microbial communities in the forest floor (Mitchell and others 2007; Aponte and others 2010a; Aponte and others 2011), all of which influence the decomposition process (Hobbie 1996; Sariyildiz and Anderson 2003; Austin and Vivanco 2006). The simultaneous effects of trees on decomposition both through their litter quality and by modifying the environmental conditions might cause positive litter-environment interactions and further increase decomposition. This interaction, termed "home-field advantage", implies litter decomposes faster beneath the tree species from which it is derived than beneath other plant covers and could be explained as an adaptation of the local soil communities to the litter produced by the plant species above them (Negrete-Yankelevich and others 2008; Ayres and others 2009). Despite the implications for ecosystem functioning and carbon cycling, the environment effect of tree species on litter decomposition has barely been explored and the relative importance of the litter versus the tree species environment effect on the decomposition process still remain unclear (but see Hansen 1999; Hobbie and others 2006; Vivanco and Austin 2008).

The litter decomposition process is ultimately driven by specific controlling factors related to the requirement of the decomposer community and whose availability is partly determined by tree species. As litter decomposition progresses through time litter quality varies and the factors controlling litter mass loss might change (Berg and McClaugherty

2008). Early decomposition is often determined by the availability of limiting elements such as N and P whereas in late stages carbon loss has been related to elements required to decompose recalcitrant components such as lignin that accumulate in the remaining litter (Güsewell and Gessner 2009; Berg and others 2010). Thus variables controlling the early decomposition stage and nutrient release could differ from those influencing the proportion of slow decomposing litter and therefore the build up of soil organic matter and carbon sequestration. Occasionally, the same variable could have counteractive effects on the early and late stages of decomposition (Berg and McClaugherty 2008; Hobbie and others 2012). For instance litter N is positively related to initial decomposition rates (Melillo and others 1982), but negatively related to the late stages of decay (Berg and Ekbohm 1991). Although the factors controlling decomposition have commonly been identified in studies addressing either the early or late decay stages, few studies have followed the changes in rate-regulating factors over the same long-term experiment.

The decay patterns of chemical elements in decomposing litter dynamics are highly diverse, even for litters of a similar type and often reflect the requirements and availability of nutrients to the decomposer community (Swift and others 1979; Staaf and Berg 1982). Limiting nutrients occurring in suboptimal amounts would be accumulated by the decomposers whereas nutrients exceeding the needs of decomposers would be released (Laskowski and others 1995). The analysis of the amounts and concentrations of nutrients along the decomposition process of different species of litter can reveal changes in the limiting elements over time, reflecting changes in the decomposition stages and processes and showing the differences in species nutrient cycling.

We aimed to compare the effects that tree species exert on litter decomposition via litter quality and via environmental conditions and to evaluate whether the factors mediating these effects change over time by studying the leaf litter decomposition and nutrient release of two

co-occurring oak species: the evergreen *Quercus suber* and the winter deciduous *Q. canariensis*. We previously demonstrated that these species generate significantly different biotic and abiotic environments beneath their canopy though their distinct leaf litter nutrient return (Aponte and others 2010a; Aponte and others 2010b; Aponte and others 2011). We studied litter decay using litterbags with single and mixed species litter because the effects of individual species may differ in mixed forest conditions as a result of positive, negative or neutral interactions between litter types (Gartner and Cardon 2004; Hättenschwiler and Gasser 2005). Litterbags were incubated in four microsites: beneath the two oak species, under shrubs and in open areas.

Our specific objectives were four: 1) To investigate the tree species effect on decomposition via litter quality both in single and mixed species conditions. 2) To evaluate tree species effect on decomposition via the distinct environment they generate beneath their canopy. We also tested for a positive litter-environment interaction supporting the home-field advantage hypothesis. 3) To identify the litter and soil chemical properties that best predicted the decay parameters associated with different stages of the decomposition process. 4) To analyze the patterns of liberation and immobilization of chemical elements from the decomposing litter of the two oak species.

METHODS

Study area

This study was conducted in the Aljibe Mountains, near the Strait of Gibraltar, southern Spain. The bedrock is dominated by Oligo-Miocene sandstone that produces acidic, nutrient-poor soils (Palexeralfs), which are frequently interspersed with layers of marl sediments that yield soils richer in clay (Haploxererts; nomenclature follows Soil Survey Staff 2010). The climate is sub-humid Mediterranean, with a dry and warm summer period of 3-4 months and

most rainfall (95%) occurring from October to May (Anonymous 2005). The dominant vegetation is a mixed forest of evergreen cork oak (*Quercus suber* L.) and winter-deciduous Algerian oak (*Q. canariensis* Willd.). These oak species differ in their leaf fall and litter quality. Leaf fall from *Q. canariensis* has a higher nutrient content (Ca, K, Mg and S) than *Q. suber*, and this difference induces distinct soil conditions via nutrient return (Aponte and others 2011). The arborescent shrubs *Erica arborea* L., *Phillyrea latifolia* L. and *Pistacia lentiscus* L. are abundant in the understorey (Ojeda and others 2000). The area has been protected since 1989 as "Los Alcornocales" (meaning "the cork oak forests") Natural Park.

Two structurally different mixed forest sites, 40 km apart, were selected within the study area. The site at San Carlos del Tiradero (hereafter called Tiradero) (36º 9' 46'' N; 5º 35' 39'' W) is located in the southern area of the Park, near the coast, at 335–360 m a.s.l. on a NE-facing slope. The mean annual rainfall is 964 mm, and the mean annual air temperature is 16.6 ºC, with a minimum of 4.1 ºC. This stand has a high density of trees (769 stems ha^{-1}), with a basal area of 47 m^2 ha^{-1}. The other site, at Sauceda (36º31'54''N; 5º34'29''W), is located inland, in the northern area of the Park, at 530–560 m a.s.l. on a NW-facing slope. It has a mean annual temperature of 15.5 ºC, with a minimum of 1.8 ºC, and a mean annual rainfall of 1470 mm. The tree density at Sauceda is relatively low, with 219 stems ha^{-1} and a basal area of 22 m^2 ha^{-1}. The two oak species, *Q. canariensis* and *Q. suber*, co-occurred at both forest sites (Pérez-Ramos and others 2008).

Litter decomposition experiment

Freshly senesced leaves of the two oak species were collected from a large forest tract near one of the sites (Sauceda) to minimize within species litter chemistry heterogeneity. The leaves were obtained by gently shaking the tree branches. The collections were made at the end of March (for *Q. canariensis*) and June (for *Q. suber*) 2007, during the respective leaf-

fall periods of the two tree species. Litter was air-dried and stored at room temperature. We prepared 11 x 11 cm litterbags (2 mm fibreglass mesh) with approximately 2.00 g of air-dried leaf litter of a given species or an equivalent mixture of the two species. The exact litter weight of each bag was recorded in grams with an accuracy of two-decimal places. Six litter bags of each species were dried at 65°C for 48h and weighed to determine the dry mass conversion that was used to calculate the initial dry mass of each sample. The bag size was consistent with the average size of *Q. canariensis* (7.4 x 3.7 cm) and *Q. suber* (4.1 x 2.4 cm) leaf litter. The mesh size was chosen to optimize access by organisms to the litter while minimizing particle loss (Karberg and others 2008). We placed the litterbags beneath the canopy of six adult individuals of *Q. suber* and six of *Q. canariensis* at the two forest sites (that is, 4 types of microsite). The footprint of a tree species on the soil is expected to be more intense within the vertical projection of the canopy (Finzi and others 1998a; Bennett and others 2009), particularly if canopies are segregated, as is the case in Sauceda. The trees selected had their closest heterospecific neighbour at a distance of 8 m in Sauceda and at 3 m in Tiradero. In addition, at Sauceda, we located litterbags in two other types of microsites (with 6 replicates each): under shrubby cover and in forest gaps with herbaceous vegetation. Litterbags were placed on the surface of the standing litter layer and fastened to the soil with 15cm long wooden sticks. In all, 432 litterbags (3 litter types x 6 types of microsites x 6 replicates x 4 harvests) were placed in the field in November 2007 and harvested every 6 months for 2 years. On each occasion, six replicate litterbags of each litter and microsite type were collected.

Upon harvest, the litter was removed from the bags, separated from roots and large soil aggregates, dried (65 º C, 48 h) and weighed. The weight of the remaining biomass was corrected for the water content of the initial air-dried samples. The leaves from the two species in the mixed litterbags were carefully separated and were treated independently

thereafter. Subsamples of the initial leaf litter from each species and the harvested litter samples were ground and analyzed for C and N content (using a Leco TruSpec analyzer) and for the total concentration of several nutrients (Ca, K, Mg, P, S, Mn, Cu and Zn) by acid digestion followed by ICP-OES (Varian 720-ES) determination to asses changes in nutrient content over time. The proportion of remaining carbon (RC) was calculated by dividing the amount of carbon at any harvest date (C concentration per g of remaining litter at that time) by the initial amount of carbon (initial concentration per g of initial litter).

Microsite soil characterization

Several inorganic and biological properties of the soils beneath the selected trees (Table 1) had been previously determined in our parallel studies of element cycling (Aponte and others 2011) and soil microbial biomass (Aponte and others 2010b). Briefly, the methods used were as follows. In November 2006, soil cores 25 cm deep were extracted with a cylindrical auger at each microsite (6 replicates per type of microsite). We determined soil pH in a 1:2.5 soil:H_2O solution. The available soil P was estimated using the Bray-Kurtz method. The soil NH_4^+ was extracted with KCl (2 M) and determined by steam distillation. The total concentrations of several nutrients (Ca, K, Mg, P, S, Mn, Cu and Zn) were determined by acid digestion followed by ICP-OES analysis (Sparks 1996). In addition, in May, September and December 2007 we sampled 8-cm-deep soil cores at the same microsites to estimate gravimetrical water content and to determine microbial C, N and P using a chloroform fumigation-extraction procedure (Brookes and others 1985; Vance and others 1987). For simplicity we use here the values of May 2007, which showed the largest variability between microsites. These measurements were used to characterize the incubation sites and determine the best predictors of litter decomposition.

Data analysis

We fitted litter change over time with two alternative decay models proposed by Wieder and Lang (1982): a single-exponential decomposition model, $M_t=e^{-k_e t}$, where M_t is the proportion of remaining biomass at time t and k_e is the decay rate, and an asymptotic model, $M_t=m + (1 – m)e^{-kt}$ where M_t is the proportion of remaining mass at time t, m is the fraction of the initial mass with a decomposition rate of zero (that is, the asymptote) and k is the decomposition rate of the remaining fraction (1–m). The asymptotic model implies that there is a limit value (m) for mass loss. This value corresponds to a very stable fraction of the litter that decomposes extremely slowly over the time span of the experiment (Berg and others 2003). In this study we have used carbon instead of biomass data to analyze decay rates, and thus avoid the confounding effects of the interactions between litter and mineral soil. All models were fitted using nls (nonlinear least squares) function in R freeware (http://www.r-project.org/) and they all constrained the proportion of initial mass (carbon) remaining at time zero to be 1. Model selection was performed using Akaike´s Information Criterion (AIC). Models whose AIC values differed by less than 2 were considered to have an equivalent ability to describe the data.

The dynamics of the element concentrations during decay were analyzed using a polynomial regression model ($Y=B_o + B_l kt + B_2(kt)^2$) that allowed both the linear and the curvilinear relationships between the chemical elements to be tested (Laskowski and others 1995). Y represents the concentration of the element at time t. The parameters B_1 and B_2 would be interpreted in terms of linear or nonlinear (unimodal or U-shaped) relationships, respectively. We used Standardized Time Units (1 STU=k years) by multiplying time by the decomposition constant k for every litter type (Laskowski and others 1995). This approach allowed us to relate the concentrations of chemical elements to the stage of decomposition rather than to absolute time and thus to compare the dynamics of chemical elements in litters having different decomposition rates. The change in the relative amount of chemical elements

during litter decomposition was calculated by dividing the amount of the element in the litterbags at any harvest date (mg of element multiplied by the g of remaining litter at that time) by the initial amount of the element (initial concentration multiplied by the g of initial litter).

We used a t-test to evaluate the differences between the forest sites in the decomposition variables (RC, chemical element concentration) and parameters (k, m, B_o, B_1 and B_2). Because the forest site had a significant effect, we used the analysis of covariance (ANCOVA) to investigate the effects of microsite and litter type on the decomposition parameters and included forest site as a covariate. Due to the unbalanced design, we first ran the analysis including only the common microsite types (understorey of *Q. canariensis* and *Q. suber*) of the two forest sites, and we then analyzed the differences between the microhabitats within each site. Post hoc comparisons were made using the Fisher LSD test. Type I error inflation resulting from repeated tests was controlled using a false discovery rate procedure (FDR), as recommended by García (2003).

To test for interactions between litter types, that is, non-additive effects of the species litter mixture on decomposition, we evaluated whether the categorical factor of individual versus mixed species (mixed) explained a significant fraction of the variability of the parameter dataset, assuming that the decay parameters from the mixed-species litterbag could be predicted from the individual species. Additionally, we compared the decomposition parameters for the individual and mixed-species litters using ANOVA. To evaluate the home-field advantage hypothesis, the litter-environment interactions were tested using the individual litter species and locations (home and away) as factors.

The best explanatory variables for the parameters associated with both the early and the late stages of the decomposition were assessed using a model-selection approach. We fitted

uni-, bi- and trivariate mixed models using the measured soil properties and litter chemical composition (determined on litter samples harvested after 6 months of incubation) as predicting variables and the forest site as random variable. The alternative models were compared using the Akaike's information criterion (AIC). The model having the lowest AIC value was selected. This model retained the predictors that were significantly related to the response variable. The R^2 value was used as a measurement of the goodness of fit of each alternative model. The conditional R^2 associated with each predictor term was calculated to evaluate the variability explained solely by each predictor. Additional models were fitted by adding the categorical variables litter type and microsite to the selected models to test for significant unmeasured effects.

RESULTS

General trends in carbon loss

The loss of leaf litter carbon showed a general exponential trend. This trend varied with the leaf litter species, the type of microsite where the litter was incubated and the general conditions of the forest experimental site (Figure 1). According to the AIC, the asymptotic model generally provided a better fit than the single-exponential model, both for models fitted to each replicate separately (74% of 144 models fitted) and for models fitted to the pooled microsite replicates (six replicates combined; 92% of 24 models fitted). In no case did the single-exponential model furnish the single best fit. The exponential decay rate was significantly correlated with the asymptote (m) ($r = -0.4$; $p < 0.001$) but not with the asymptotic decay rate ($r = 0.08$; $p < 0.30$). The asymptotic model will be used hereon and, for simplicity, we will refer to the asymptotic decay rate as decay rate (k).

Litter-type effects on carbon loss

Leaf litter species determined significant differences in the remaining carbon (RC) during the first year ($p<0.001$), when the RC in *Q. suber* litter was higher (62.9% vs. 55.6%) than in *Q. canariensis* (Figure 1, Supplementary Figure S1). However, both oak species converged to similar carbon values during the second year. We observed no interaction between species litter, that is, each species showed similar RC values in single and mixed conditions throughout the two years ($p>0.05$). The decomposition rate (k) was higher for *Q. canariensis* litter than for *Q. suber* litter both in single (2.01±0.08 vs. 1.14±0.07; $p<0.0001$) and mixed litter conditions (1.99±0.11 vs. 1.28±0.09; $p<0.0001$), indicating a faster initial decomposition for litter of the deciduous *Q. canariensis*. However, the limit value (m), representing the fraction of slowly decomposable biomass at the later stage of decomposition, was also higher for *Q. canariensis* than for *Q. suber* litter (0.40 ± 0.01 vs. 0.31 ± 0.02, $p<0.0001$) when incubated in single species conditions. No differences were found in the limit value in the mixed species litter (0.37 ± 0.02 vs. 0.33 ±0.02, $p<0.474$) (Figure 2).

Environment effect on carbon loss

The microsite environment where litter was incubated had significant effects on the litter remaining carbon, particularly at the Sauceda forest site (Supplementary Figure S2) and for the litter of the deciduous species, *Q. canariensis*. The decomposition rate of *Q. canariensis* litter beneath *Q. canariensis* trees (k=1.69) was significantly lower than beneath *Q. suber* (k=2.45); thus after the first 6 months, the RC beneath *Q. canariensis* (64.2%) was higher than beneath the *Q. suber* (57.2%; $p<0.0102$). A similar but not significant difference occurred for the *Q. suber* litter, which tended to decompose slower (higher RC) beneath *Q. canariensis* canopy (70.79±0.01% vs. 68.12±0.01%). Opposite patterns were observed after 24 months of incubation, when the RC of *Q. canariensis* litter was higher beneath *Q. suber* (41.49±0.02% vs. 34.68±0.03%) as it was the fraction of slowly decomposable carbon, that is, the limit value (0.34 ± 0.01 vs. 0.31±0.02), although the differences at this time were not

significant. Among all the microsites studied, the litter incubated beneath the shrubs showed the highest decomposition rate ($k=1.82$, $p<0.05$) and the highest limit value ($m=0.42$; $p<0.009$). The lowest limit value was found in the open areas ($m=0.29$, $p<0.036$).

There were no positive interactions between the litter species and the environment where litter was incubated (microsite type) either for the remaining carbon or for the decay rate. On the contrary, at Sauceda the decay rate of *Q. canariensis* litter was significantly lower under the trees of the same species than in other incubating environments ($p<0.022$, Figure 2). A similar but insignificant interaction was observed in Tiradero. Therefore the field-home advantage hypothesis was not supported by these data.

Differences between forest sites in decay rates

The average proportion of remaining carbon after the two-year decomposition period differed significantly between the two forest sites (F: 112.829; $p<0.000$), with 39% (range 13-60%) of the carbon remaining in Sauceda and 46% (range 34-66%) in Tiradero (Supplementary Figure S2). The two sites also exhibited different limit values (Sauceda: $m=0.34\pm0.01$; Tiradero: $m=0.39\pm0.01$; $p<0.008$), but similar decay rates (Sauceda: $k=1.63\pm0.07$; Tiradero: $k=1.55\pm0.09$; $p<0.5$).

Leaf litter decay and nutrient dynamics

The initial concentrations of Ca, Mg, N, P and S were higher in *Q. canariensis* than in *Q. suber* leaf litter, whereas those of C and Mn were higher for *Q. suber* (Table 2). In particular, Ca and Mg had approximately 1.5-fold higher values in the litter of *Q. canariensis*. The patterns of nutrient immobilization and release over time differed among elements as revealed by the changes in their concentrations (Figure 3, Supplementary Table S1) and amounts (Figure 4). The polynomial model fitted to the N and Ca concentrations showed a

unimodal time course, with an initial period of increasing concentration followed by a period of element loss. The curves for Ca concentration were approximately parallel for both oak species. Those for N converged at the latter stages of decomposition, owing to an increased N concentration in the *Q. suber* litter. The concentration of Mg remained relatively constant with time for both species. The litter P content decreased linearly for *Q. canariensis* but remained constant for *Q. suber*. Approximately 80% of the K was lost in the first six months (Figure 4) matched by a strong decrease in its concentration (Figure 3, Supplementary Table S1). The concentrations of Zn and Mn showed monotonic increases. The B_0 values for the two litter types differed significantly for all the chemical elements studied, whereas differences in the parameters B_1 and B_2 were found for Ca, P, Mn and Zn (Supplementary Table S1). The differences in element net loss between the litter types indicated a higher and faster nutrient release (for Ca, Mg, P and S) from *Q. canariensis* litter (Figure 4). Nitrogen showed a distinctively different release pattern for the two oaks, being relatively immobilized in *Q. suber* litter but released from *Q. canariensis* litter. Calcium was immobilized during the first 6 months in *Q. canariensis* litter, but longer (12 months) in *Q. suber* litter.

The microsite type had no effect on any regression parameters. However, it affected chemical element concentration and element abundance. These values were generally higher beneath *Q. canariensis* and shrubs than beneath *Q. suber* and herbs (See Supplementary Figure S3). We found no interactions between species in the mixed litterbags, that is, the parameters B_0, B_1 or B_2 did not differ between the individual and mixed-species litter for any chemical element.

Predictors of litter decomposition

Both litter type and microsite environment affected decomposition parameters although the relative magnitude of their effect (measured as the conditional R^2) differed and changed over

time. On average, microsite (as a categorical predictor) significantly explained 4.4% of the variance of the parameters related to early (3.4% of k and 5.3% of RC at 6 months) and 4.5% of the variance of the parameters related to late decomposition (3.7% of m and 5.2% of RC at 24 months). The variance explained by litter type decreased from early (35.2% of k and 28.4% of RC at 6 months) to late (15.9% of m and not significant for RC at 24 months) decomposition parameters.

Different litter and soil variables emerged as the best predictors for decomposition parameters (Supplementary Table S2). Five elements, namely N, Ca, S, P and Mn, and the soil moisture content came out as the best predictors for decomposition. Most of these predictors influenced both early and late decomposition, of which soil P (as total P or microbial P) and soil moisture positively influenced both early and late decomposition whereas litter N (and the related stoichiometric ratio C:N), litter Ca and soil N had counteractive effects on early and late stages. Litter with higher N and Ca content had a faster early decomposition but a higher fraction of slowly decomposable carbon. Incubation in soils with high N content was related to lower decay rates but lower limit values (Figure 5). Litter Mn and soil S best predicted the remaining carbon at 24 months and the decomposition limit value (m). They were positively related with carbon loss at latter stages but showed no effect on early decay parameters.

DISCUSSION

Our results revealed that tree species can affect decay dynamics both by their different litter quality and by the different environmental conditions underneath. The effect of litter type on the decomposition process decreased over time, but it was invariably more important than the effect associated with the environmental conditions. We found no positive litter-environment interaction that would support the “home-field advantage” hypothesis. Among

the main decay controlling factors we can distinguish three types: variables that positively influenced litter decay through the early and late decomposition stages, variables that exerted a counteractive effect during early and late decomposition, and variables that only affected the late decomposition stage. Our analysis on the dynamics of nutrient loss revealed that the initial nutrient content of leaf litter differed between tree species and had a cascade effect on the rate, proportion and amount of nutrient loss, thus underpinning the tree species effect on nutrient cycling.

Decomposition as a two-stage process

The studied oak litter decomposition best fitted an asymptotic model. This model assumes that there is a fraction of plant litter that decomposes at a very slow rate, the reason being the increased concentration of recalcitrant substances such as soluble and non-lignified carbohydrates that are degraded during the early stages of decomposition (Berg and McClaugherty 2008). Although the asymptotic model has provided a better fit than the single-exponential model, in decomposition studies the latter is more widely used (and criticised; see Wieder and Lang 1982; and Ostrofsky 2007). The explicit differentiation between early and late decomposition stages has allowed us to reveal that the factors controlling leaf litter decomposition and carbon cycling in the studied forests change through time.

Litter quality effect on decomposition changes over time

One of the most important findings of this study is that as decomposition progressed over the two-year experiment, the relative importance of the effect of the litter type decreased and the direction of its effect reversed. In particular, the deciduous oak´s litter decayed faster in early stages but the perennial oak´s litter decayed further in late stages (Figure 1). Litter N and Ca

were positively related to litter decay during the initial period of decomposition but they were negatively related to carbon loss during the late decomposition stage, thus revealing a shift in their effect on the decay process over time. During the decomposition of leaf litter, a vast array of chemical, physical and biological agents act upon litter constituents changing their compositions and concentrations (Berg and McClaugherty 2008). As litter quality changes, so does the influence of rate-determining litter chemical components. Berg and others (2000) proposed a three-phase decay model with an early decomposition stage, when the rapid decay of soluble and non-lignified carbohydrates is regulated by N, P and S contents, a late decomposition stage, when decay is regulated by the degradation of lignin, and a final or "humus-near" stage. The turning point between the early and late stages of decomposition is often encompassed by a peak in Ca immobilization followed by a loss indicating the onset of net lignin degradation (Berg and McClaugherty, 2008). Litter N has often been identified as a rate-enhancer factor for early decomposition (Gallardo and Merino 1993; Berg 2000; Hobbie and others 2012). The litter C:N ratio, as an index of the nutritional balance, has also been found to affect microbial activity and regulate the nutrient dynamics of the litter (Enríquez and others 1993; Güsewell and Gessner 2009). However high initial litter N concentration also suppresses lignin-degradation rates by hindering the formation of lignolityc enzymes in the population of lignin degrading organisms (white rot fungi) thus impeding litter decomposition in the late stage (Eriksson and others 1990; Hatakka 2005). Our study reveals that litter N can reverse its effect from rate-enhancer to rate-retarding in a two-year period.

Previous studies have shown a strong and positive relationship between litter Ca and decomposition rates in temperate forests (Chadwick and others 1998; Hobbie and others 2006). Calcium supports the growth of white rot fungal species and is an essential cofactor of the lignin-degrading enzymes of the decomposer microflora (Eriksson and others 1990). The emergence of litter Ca as a predictor of early decomposition together with the concentration

and immobilization patterns observed in this study suggests that degradation of lignin is already important in this early stage of decomposition. Davey and others (2007) reported an early onset of lignin degradation on *Quercus robur* litter indicated by a significant correlation between decay rate and essential lignin degrading co-factors such as Ca and Mn. Litter Ca has been related to increased microbial activity, fungal and earthworm abundance and diversity and forest floor removal rates (Berg and others 2003; Reich and others 2005; Hobbie and others 2006; Aponte and others 2010a). Due to the role of Ca in lignin decomposition, we expected a positive relation between litter Ca and mass loss throughout the decomposition process, as it was previously described for litter of temperate and boreal trees (Berg and others 1996; Berg 2000). However our results showed a counteractive effect of Ca during early and late decomposition stages, which had been also observed by Davey and others (2007) on *Quercus robur* litter. They suggested that Ca contributed to a percentage of the recalcitrant fraction of the litter, thus leaves with a higher Ca concentration (that is, *Q. canarienis* in this study, Figure 3) would have a higher decay rate because of the lignolytic effect, but also a higher fraction of non-decomposable mass.

The role of leaf litter Mn

Litter manganese, which was 25% higher in the perennial leaf litter, was the most important rate-controlling factor during late decomposition, thus leading to an unexpectedly higher carbon loss from the perennial than the deciduous litter. There is contradicting evidence for the role of Mn during late decay stages. Berg and others (2007) showed that the Mn concentration in the litter of five conifer species (range of 0.04 – 7.69 mg g^{-1}) positively affected the loss of litter mass at very late decomposition stages (up to 5 years), provided that the Mn concentration of the litter was sufficient (> 2 mg g^{-1}). On the contrary, Davey and others (2007) found that litter Mn was not related to the limit value of decomposition of oak

litter, but it was positively correlated to the early decay rate. Manganese is essential for the activity of Mn peroxidase, a lignin-degrading enzyme (Perez and Jeffries 1992). Interestingly, our results differ from the above in that Mn showed no significant effect on early decomposition but it was the most important rate-controlling factor after only two years, despite having a low initial concentration (average of 1 mg g^{-1}) and a relatively restricted concentration range (0.66 – 1.27 mg g^{-1}). We have shown that certain litter nutrients, that is, N, Ca and Mn, exert different effects on determining litter decomposition over time, highlighting the importance of addressing all stages of decomposition when studying the factors controlling carbon cycling and revealing that litter that initially decomposed faster might as well generate the largest pool of accumulated carbon.

Tree species' environment effect on decomposition changes over time

Differential tree species environment significantly influenced decomposition although the magnitude of this effect was smaller than the litter type effect and it mostly affected the deciduous litter decay. The effect exerted by the tree species environment also reversed during the decomposition process (like the litter type effect), but in this case the pattern was the opposite. Decay beneath the deciduous oak, where soil was richer in nutrients, tended to be slower during the early stage but to proceed further during the late stage. Soil N and P, and soil moisture were the variables best related to litter decay. The role of soil nutrient availability on litter decomposition processes is still poorly understood with most studies focused on litter nutrients (Davey and others 2007; Strickland and others 2009; Berg and others 2010). Soil N was negatively related to initial decay rate while it promoted an extended decomposition in the late stage. The effect of exogenous N on litter decay has been

studied in naturally occurring gradients and experimental conditions (for example, McClaugherty and others 1985; Hobbie 2008; Hobbie and others 2012) but the observed effects have been inconsistent. Higher N availability sometimes increased initial decay rates although most often it had a negligible or even negative effect on decomposition (Prescott 1995; Hobbie and Vitousek 2000). These studies suggest that the soil N effect on decay rates depends on the quality of the decomposing litter (McClaugherty and others 1985; Hobbie and Vitousek 2000; Hobbie and others 2012). We can hypothesize that during early decomposition, higher N availability could hinder the decay of the already N-rich deciduous litter by negatively affecting the N-sensitive fungi that participate in lignin degradation. This effect would be subdued for the N-poor perennial litter. As decay progresses to later stages and litter N concentration decreases, the external N concentration may have a positive influence on the general activity of the microbial community and thus promote a higher cumulative mass loss. This hypothesis would also underpin the observed negative interaction between litter and environment, that is, the deciduous leaf litter decomposed faster in environments other than its own. This interaction was contrary to what was expected under the home-field advantage hypothesis (Vivanco and Austin 2008; Ayres and others 2009).

Both soil P (either as C:P, total or microbial P) and soil moisture exerted a relatively small but constant positive influence on litter decomposition, suggesting a limiting role of these variables for decomposers activity. In a chronosequence study, soil P was negatively correlated with the amount of accumulated carbon in forest soils (Vesterdal and Raulund-Rasmussen 1998). In the same studied forest, soil P and soil moisture were found to be key factors controlling soil microbial biomass (Aponte and others 2010b). To date few studies have investigated the influence of tree species on decomposition via the environmental conditions they generate (Hobbie and others 2006). Our results suggest that the magnitude of

tree species effect varies depending on the litter quality and soil conditions, thus inviting further exploration of the circumstances that would magnify this effect.

Nutrient loss rates differed between litter types

Chemical elements differed in their litter decomposition dynamics although all the chemical elements (except Mn and Cu) exhibited similar relative mobility in the two litter types. On average, the elements were released in the order K>Mg>C>P>Mn>S>N>Ca>Cu>Zn (Figure 4). Some patterns of litter nutrient release described here are similar to those from other temperate forests: the rapid release of K is typically reported from a broad range of forest ecosystems (Attiwill 1968; Berg 1986; Blair 1988), and the increasing concentration and immobilization of Zn has been related to throughfall input (Laskowski and others 1995). In contrast, other elements have shown particular dynamics in this studied forest. For example, in other studies P is immobilized at the initial stages of decomposition and subsequently released (Staaf and Berg 1982; Maheswaran and Attiwill 1987). However, this immobilization phase did not occur in this experiment. Other studies showed continued loss of Ca, Mg and Mn, but the patterns reported here were different. In general, distinct patterns in the dynamics of particular chemical elements in various forest ecosystems reflect the different availabilities of nutrients to decomposers. Thus those elements with concentrations below the limiting threshold for decomposers would be immobilized in litter (Swift and others 1979; Staaf and Berg 1982). We have observed that N and Ca, early rate-enhancer factors, were immobilized in the litter during the early decomposition stages whereas Mn was immobilized during the late stages of decomposition. These temporal patterns reflect the changes in the factors controlling decay as decomposition progresses, litter quality changes and decomposer requirements vary.

An important contribution of this study to understanding the tree species effect on decomposition and ecosystem properties was to reveal that, although the patterns of nutrient concentration during the decomposition process were similar for both oak species, the patterns of net nutrient release differed. The litter produced by the deciduous oak had a higher initial nutrient content and released its nutrients at a higher rate and in a higher proportion than the litter of the perennial oak species thus inducing elevated fertility beneath its canopy and faster nutrient cycling compared to the perennial species. The contrasting effect of deciduous and perennial species on soil fertility and nutrient cycling has been addressed in many correlational and descriptive studies (Hobbie 1992; Finzi and others 1998b; Augusto and others 2002; Aponte and others 2011). Our results explicitly revealed one of the potential mechanisms underlying that effect.

CONCLUSIONS

This study has provided new insights into the factors controlling the decomposition process demonstrating the importance of the effect that tree species have on the litter decay rate, carbon accumulation and nutrient cycling. Our results showed that tree species affected decomposition mostly through their litter quality and to a lesser extent through the differential environmental conditions they generated beneath their canopy. More importantly by using an asymptotic model that explicitly distinguishes between the early and late decomposition stages we have been able to demonstrate that the rate-controlling factors vary and reverse their effect over time. Such changes suggest that the limiting elements vary as decomposition proceeds and litter quality decreases. The deciduous oak species (*Q. canariensis*) initially decomposed faster but had a higher fraction of slowly decomposable mass than the coexisting perennial oak (*Q. suber*), therefore producing a larger pool of accumulated stabilized carbon. This implies that the initial litter decay rate and decomposition limit value might be uncoupled and thus litter that decomposes slower could

also decompose further and have a lower capacity for carbon sequestration. The differences observed in the nutrient release between the two oak species reveal a potential mechanism underlying their distinct effects on nutrient cycling. For most macronutrients (N, Ca, Mg, P and S), the net nutrient release was higher for the deciduous oak, which showed the highest initial nutrient concentrations and highest proportion of nutrient released. These conditions fostered soil fertility and generated an environment that further influenced the decay process. We have presented here a comprehensive study on the tree species effect on litter decomposition and provided a better understanding of the complexity of the factors controlling decay rates and carbon accumulation from a temporal perspective. Our results contribute to a better understanding of the effect of tree species on ecosystem functioning and will guide future work on the decomposition process in other ecosystems.

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FIGURE LEGENDS

Figure 1. Predicted variation in the remaining carbon (%) of leaf litter with time as a function of forest site and oak species, using the fitted asymptotic model.

Figure 2. Decomposition constants (mean+SE) of the single (C-*Q. canariensis*, S-*Q. suber*) and mixed (MC- *Q. canariensis*, MS- *Q. suber*) litters (*** $p<0.001$, ** $p<0.01$, *$p<0.05$, ns not significant).

Figure 3. Dynamics of the concentration of chemical elements in the decomposing leaf litter of *Q. canariensis* (solid line and filled circles) and *Q. suber* (dashed line and hollow circles). Error bars indicate 95% CI. Time is expressed in standardized time units (STU=time (yr) x decomposition constant k).

Figure 4. Dynamics of the net immobilization of elements in the decomposing litter of *Q. canariensis* (solid lines and filled circles) and *Q. suber* (dashed lines and hollow circles) during the 2 year experiment. Values are relative to initial element abundance.

Figure 5. Variation of the asymptotic decay rate (k, filled circles) and the limit value of the decomposition (m, hollow circles) in relation to the N concentration in the soil and litter of the studied oak trees. Increasing decay rate indicates a faster early decomposition while increasing limit value indicates a higher fraction of slowly decomposable litter.

TABLES

Table 1. Description of the Soil Beneath the Oak Trees where Litterbags were Incubated in the Two Studied Forests

		Sauceda				Tiradero			
		Q. canariensis		*Q. suber*		*Q. canariensis*		*Q. suber*	
pH		5.85	(0.17)	5.26	(0.38)	4.88	(0.24)	4.61	(0.14)
$N\text{-}NH4^{+}$	(mg kg^{-1})	22.3	(11.9)	30.3	(8.0)	4.6	(3.4)	2.8	(0.9)
$P\text{-}PO4^{-}$	(mg kg^{-1})	3.31	(0.97)	4.89	(3.56)	3.02	(1.35)	1.76	(1.04)
N	(%)	0.28	(0.04)	0.22	(0.02)	0.26	(0.11)	0.22	(0.06)
Ca	(mg kg^{-1})	3354	(839)	2369	(756)	1348	(1161)	503	(287)
K	mg kg^{-1})	3531	(954)	3977	(1266)	1340	(903)	1501	(460)
Mg	(mg kg^{-1})	3608	(785)	3542	(698)	1176	(592)	1223	(337)
P	(mg kg^{-1})	294	(65)	279	(37)	219	(66)	229	(44)
S	(mg kg^{-1})	251	(56)	216	(13)	255	(40)	238	(43)
Sand	(%)	45.0	(5.1)	46.9	(10.4)	63.0	(6.6)	62.2	(5.9)
Loam	(%)	16.6	(3.2)	18.7	(5.4)	16.5	(3.6)	13.8	(3.0)
Clay	(%)	38.3	(4.8)	34.4	(5.9)	20.5	(4.9)	23.9	(4.9)
Soil moisture	(%)	26.6	(2.4)	25.5	(6.0)	16.3	(3.8)	15.3	(2.0)
Organic matter	(%)	16.6	(1.7)	14.8	(3.0)	11.7	(4.4)	10.5	(1.3)
Cmic	(mg kg^{-1})	1519	(382)	1035	(384)	945	(203)	929	(144)
Nmic	(mg kg^{-1})	266	(54)	161	(87)	120	(30)	116	(25)
Pmic	(mg kg^{-1})	51.0	(7.1)	50.4	(16.1)	17.4	(11.6)	14.7	(6.3)
C/N		13.8	(1.3)	16.3	(1.5)	16.8	(2.0)	17.9	(1.7)
C/P		156.5	(47.2)	227.7	(55.1)	118.7	(29.7)	197.2	(24.8)
N/P		11.3	(3.0)	14.1	(3.7)	11.4	(2.9)	11.2	(2.3)

Data taken from Aponte and others 2010b, 2011 and unpublished results. Mean (St. dev.)

Table 2. Initial Concentration (mean ± st. dev.) of Chemical Elements in Decomposing Leaf Litter

Element		*Q. canariensis*			*Q. suber*			F	P value
C	(%)	43.68	±	0.14	46.03	±	0.25	385.41	**0.000**
N	(%)	1.24	±	0.11	0.88	±	0.09	31.21	**0.000**
Ca	(g kg^{-1})	14.84	±	0.76	9.25	±	0.51	221.06	**0.000**
K	(g kg^{-1})	5.44	±	0.69	4.47	±	0.74	4.79	0.056
Mg	(g kg^{-1})	2.11	±	0.07	1.43	±	0.08	172.95	**0.000**
P	(g kg^{-1})	1.00	±	0.11	0.62	±	0.12	22.29	**0.001**
S	(g kg^{-1})	1.01	±	0.04	0.78	±	0.05	55.15	**0.000**
Mn	(mg kg^{-1})	864	±	136	1075	±	138	6.42	**0.032**
Zn	(mg kg^{-1})	22.28	±	6.51	17.05	±	6.41	2.67	0.137
Cu	(mg kg^{-1})	5.46	±	0.63	4.72	±	0.52	4.74	0.057
C/N		35.4	±	3.3	53.0	±	6.3	35.42	**0.000**
C/P		43.9	±	4.5	80.8	±	18.1	19.47	**0.002**
N/P		1.23	±	0.17	1.52	±	0.26	4.30	0.071

Differences between oak species were tested with one-way ANOVA. Significant differences are indicated by bold-face P values

Figure S1. Remaining carbon (%) observed for the single- (C-*Q. canariensis*, S- *Q. suber*) and mixed- (MC, MS) species litter at the two study sites. Differences between litter types are shown (* $p<0.05$, ** $p<0.01$, and *** $p<0.001$).

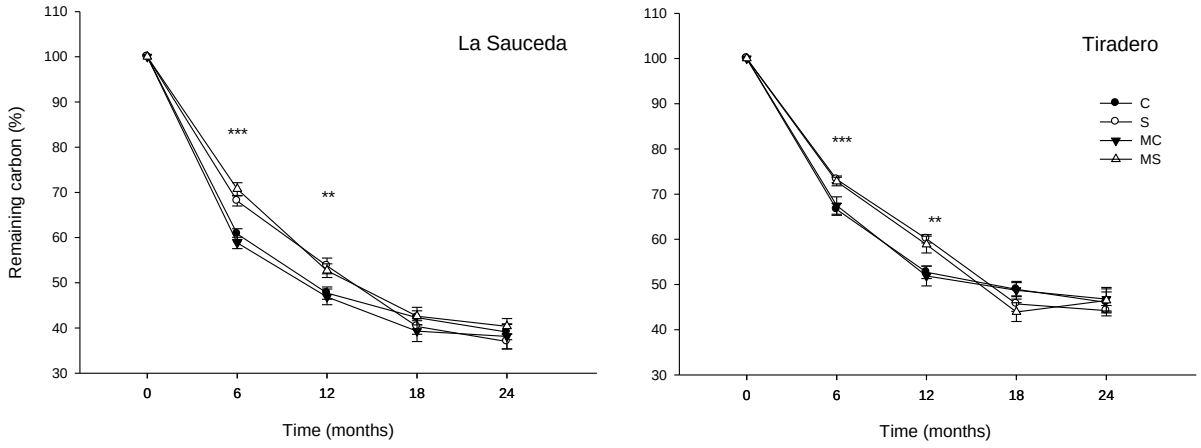

Figure S2. Remaining carbon (%) after 6 and 24 months for *Q. canariensis* and *Q. suber* leaf litter in the four microsites at Sauceda. One standard error of the mean is plotted.

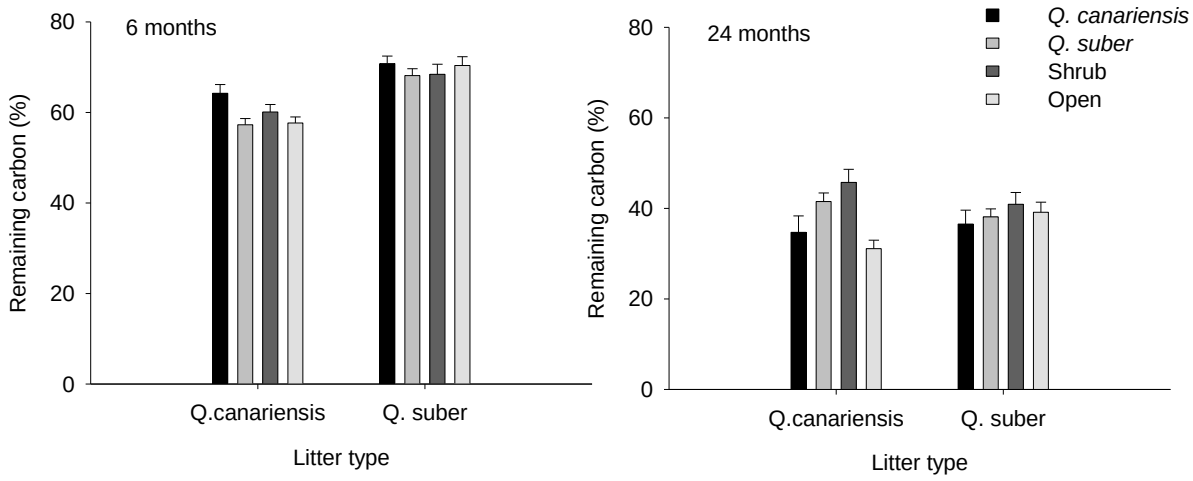

Figure S3. Average concentrations of chemical elements after the 2-year experiment for each site and microsite (*** p<0.001, ** p<0.01, *p<0.05, ns not significant).

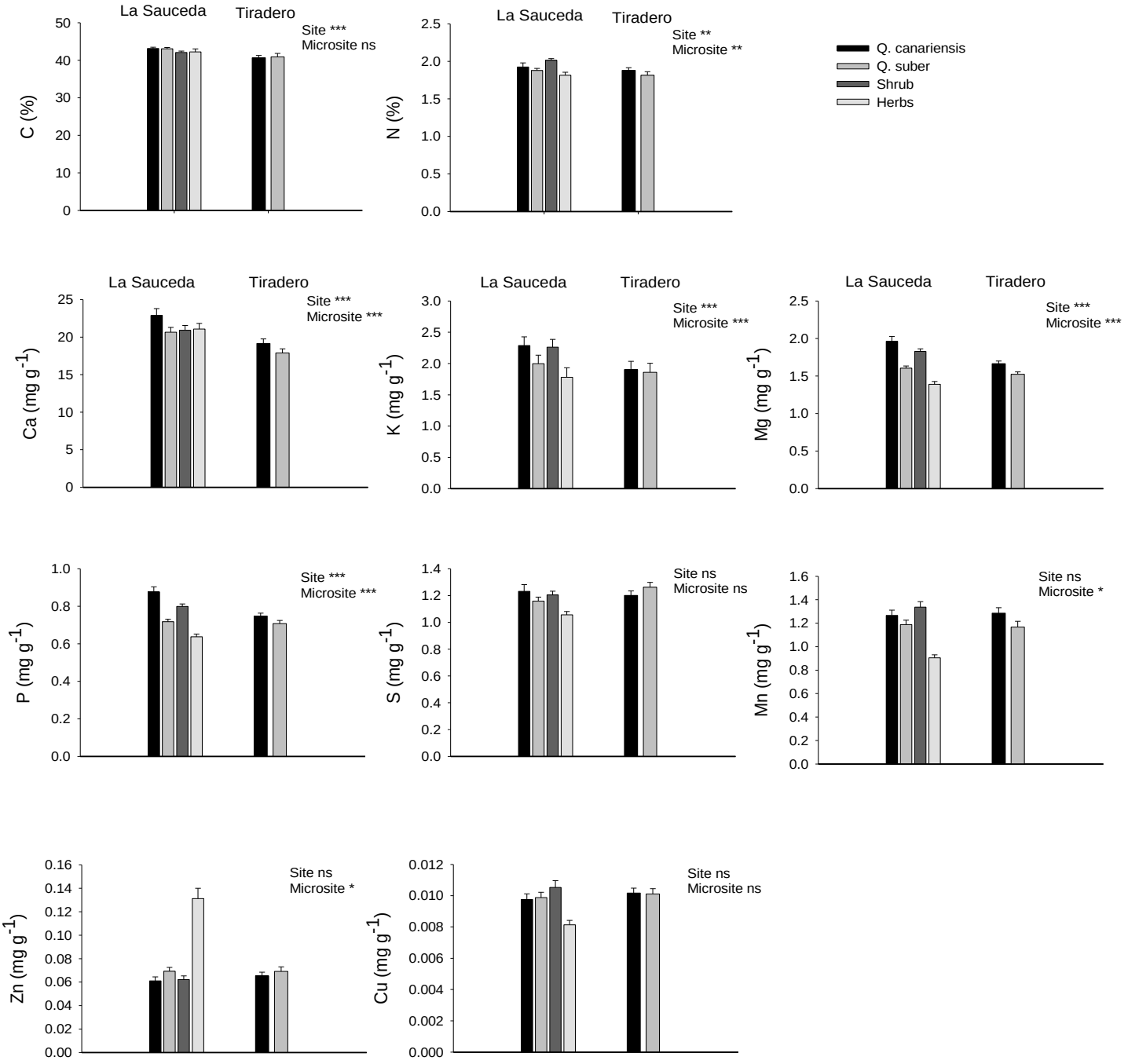

Table S1. Relation between Element Concentrations (Y) and Standardized Time by the Decomposition Constant (ST) for Leaf Litter of *Q. canariensis* (C) and *Q. suber* (S)

Element	Litter	B_o		B_1		B_2		R^2
C	C	43.87 [a] ±0.19	***	-6.82 [a] ± 0.90	***	5.20 [a] ± 0.94	***	0.19 ***
	S	46.17 [b] ±0.22	***	-3.31 [b] ± 1.13	**	-0.34 [b] ± 1.21	ns	0.24 ***
N	C	1.28 [a] ±0.02	***	1.83 [a] ± 0.09	***	-1.28 [a] ± 0.10	***	0.66 ***
	S	0.88 [b] ±0.02	***	2.20 [a] ± 0.10	***	-1.22 [a] ± 0.11	***	0.80 ***
Ca	C	16.66 [a] ±0.93	***	27.12[a] ± 3.52	***	-14.99[a] ± 2.84	***	0.38 ***
	S	9.33[b] ±0.56	***	19.50[b] ± 2.39	***	-9.09[b] ± 2.11	***	0.53 ***
K	C	4.747 [a] ±0.141	***	-9.537[a] ± 0.536	***	5.736[a] ± 0.433	***	0.73 ***
	S	3.990[b] ±0.122	***	-8.658[a] ± 0.518	***	5.962[a] ± 0.457	***	0.68 ***
Mg	C	2.106 [a] ±0.077	***	-0.258[a] ± 0.293	ns	-0.105[a] ± 0.236	ns	0.10 ns
	S	1.391 [b] ±0.055	***	-0.194[a] ± 0.233	ns	0.396[a] ± 0.206	ns	0.08 ns
P	C	0.986[a] ±0.031	***	-0.425[a] ± 0.117	***	0.166[a] ± 0.095	ns	0.20 ns
	S	0.596[b] ±0.027	***	0.184[a] ± 0.113	ns	-0.044[a] ± 0.100	ns	0.08 ns
S	C	1.058[a] ±0.057	***	1.288[a] ± 0.215	***	-1.059[a] ± 0.173	***	0.18 ns
	S	0.751 [b] ±0.046	***	1.428[a] ± 0.196	***	-0.998[a] ± 0.173	***	0.28 ns
Mn	C	0.798[a] ±0.071	***	0.895[a] ± 0.269	**	-0.318[a] ± 0.217	ns	0.20 ns
	S	1.038 [b] ±0.061	***	0.049[a] ± 0.258	ns	0.487[a] ± 0.228	*	0.25 ns
Zn	C	0.028[a] ±0.008	***	0.122[a] ± 0.031	***	-0.012[a] ± 0.025	ns	0.43 ns
	S	0.015 [b] ±0.006	*	0.117[b] ± 0.025	***	-0.033[b] ± 0.022	ns	0.40 ns
Cu	C	0.006[a] ±0.000	***	0.014[a] ± 0.002	***	-0.007[a] ± 0.001	***	0.41 ***
	S	0.005 [b] ±0.000	***	0.015 [a] ± 0.002	***	-0.01 [a] ± 0.00	***	0.58 ***

Regression model: $Y=B_o+B_{1*}ST+B_{2*}ST^2$; the significance of the parameters is indicated (*** $p<0.001$, ** $p<0.01$, *$p<0.5$, ns=not significant). Superscript letters (a,b) indicate significant differences between litter types for each element and parameter ($p<0.05$).

Table S2. Results of Model Selection for Remaining Carbon After 6 (RC_6) and 24 Months (RC_{24}) and Values of Decay Parameters (k and m)

Response variable	Variable 1	Con. R^2	Variable 2	Con. R^2	Variable 3	Con. R^2	AIC	BIC	R^2	AIC null	Litter p (χ^2)	Litter Con. R^2	Microsite p (χ^2)	Microsite Con. R^2
RC_6	Litter C:N (+) ***	26.22	Soil N (+) ***	11.32	Pmic (-) ***	4.05	-299.6	-284.3	53.83	-238.9	**	4.7	ns	-
	Litter C:N (+) ***	25.14	Soil N (+) ***	11.92	Soil moisture (-)**	3.40	-298.3	-283.0	53.21		***	5.19	ns	-
	Litter N (-) ***	23.35	Soil N (+) ***	12.52	Pmic (-) ***	3.31	-293.9	-278.6	50.97		***	7.18	ns	-
	Litter Ca (-) ***	13.04	Litter N (-) ***	8.76	Litter S (+) ***	8.54	-293.3	-278.0	52.10		ns	-	**	4.09
RC_{24}	Litter Mn (-) **	7.15	Soil S (-)**	5.31	Pmic (-)**	3.24	-207.2	-192.1	36.05	-185.0	ns	-	ns	-
	Litter Mn (-) **	6.94	Soil S (-)**	6.12	Soil moisture (-)**	2.30	-205.9	-190.7	35.11	-185.0	ns	-	ns	-
	Litter Mn (-) ***	8.41	Soil P (-)**	5.72	Soil moisture (-)*	2.58	-205.3	-190.2	34.71		ns	-	ns	-
Decay rate (k)	Litter C:N (-) ***	31.32	Soil N (-) **	5.13	Soil C:P (+) *	4.10	162.6	178.0	41.95	208.3	***	6.66	ns	-
	Litter N (+) ***	25.36	Soil N (-) **	6.11	Soil C:P (+) **	4.60	171.9	187.2	35.99		***	12.5	ns	-
Limit value (m)	Litter Mn (-) ***	10.79	Litter C:N (-) ***	9.88	Soil P (-)***	9.65	-149.5	-134.2	35.54	-117.3	*	2.7	ns	-
	Litter C:N (-) ***	13.42	Soil C:N (+) ***	8.55	Litter Mn (-) **	5.55	-147.9	-132.6	34.44		ns	-	ns	-
	Litter Mn (-) ***	16.31	Soil P (-)***	10.55	Litter Ca (+) ***	8.27	-147.2	-131.9	33.94		*	3.71	ns	-

Selection of the minimal adequate model was based on the lowest AIC value and resulted in retaining the prediction terms significantly related to the response variable and having a significant p (χ^2). The p (χ^2) values show a χ^2 comparison of models excluding the predictor term. Models whose AIC values differed less than 2 were considered to have equivalent ability to describe the data. The sign of the relationship between selected variables and response variables (- or +) and the p (χ^2) (*** <0.001, **<0.01, *<0.05, ns=not significant) are indicated. The table presents the conditional variance (Con. R^2) explained by each variable, the total variance explained by the model (R^2), the Akaike Information Criterion (AIC), the Bayesian Information Criterion (BIC), and the AIC of the null model. Additional models were fitted by adding the categorical variables litter type and microsite to the selected models to test for unmeasured effects. Both the significance of the categorical variables (p (χ^2)) and the conditional variance retained are shown. Both significance of the categorical variables (p (χ^2)) and conditional variance retained are shown.