Tree species effect on litter decomposition and nutrient release in Mediterranean oak forests change 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 promoted 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, i.e. 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 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 et al., 1982; Cornelissen et al., 2006; Hobbie et al., 2006; Cornwell et al., 2008; Güsewell and Gessner, 2009; Berg et al., 2010). But tree species can also alter decomposition rates indirectly through their 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 et al., 2007; Aponte et al., 2010a; Aponte et al., 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 et al., 2008; Ayres et al., 2009). Despite the implication 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 vs. environment tree species effect on decomposition process still remain unclear (but see (Hansen, 1999; Hobbie et al., 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 the carbon loss has been related to elements required to decompose recalcitrant components such as lignin that accumulate in remaining litter (Güsewell and Gessner, 2009; Berg et al., 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 et al., 2012). For instance litter N is positively related to initial decomposition rates (Melillo et al., 1982), but negatively related to late stages decay (Berg and Ekbohm, 1991). Whereas 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 et al., 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 et al., 1995). The analysis of the amounts and concentrations of nutrients along the decomposition process of different species 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 et al., 2010a; Aponte et al., 2010b; Aponte et al., 2011). We studied litter decay using litterbags with single and mixed species litter since 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 analyse 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 et al., 2011). The arborescent shrubs *Erica arborea* L., *Phillyrea latifolia* L. and *Pistacia lentiscus* L. are abundant in the understorey (Ojeda et al., 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 et al., 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 optimise access by organisms to the litter while minimising particle loss (Karberg et al., 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 (i.e. 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 et al., 1998a; Bennett et al., 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 analysed for C and N content (using a Leco TruSpec analyser) 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 characterisation

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 et al., 2011) and soil microbial biomass (Aponte et al., 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 et al., 1985; Vance et al., 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 (i.e., 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 et al., 2003). In this study we have used carbon instead of biomass data to analyse 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.

197 The dynamics of the element concentrations during decay were analysed using a polynomial
198 regression model ($Y=B_o + B_1kt + B_2(kt)^2$) that allowed both the linear and the curvilinear
199 relationships between the chemical elements to be tested (Laskowski et al., 1995). Y represents
200 the concentration of the element at time t. The parameters B_1 and B_2 would be interpreted in
201 terms of linear or nonlinear (unimodal or U-shaped) relationships, respectively. We used
202 Standardised Time Units (1 STU=k years) by multiplying time by the decomposition constant k
203 for every litter type (Laskowski et al., 1995). This approach allowed us to relate the
204 concentrations of chemical elements to the stage of decomposition rather than to absolute time
205 and thus to compare the dynamics of chemical elements in litters having different decomposition
206 rates. The change in the relative amount of chemical elements during litter decomposition was
207 calculated by dividing the amount of the element in the litterbags at any harvest date (mg of
208 element multiplied by the g of remaining litter at that time) by the initial amount of the element
209 (initial concentration multiplied by the g of initial litter).

210 We used a t-test to evaluate the differences between the forest sites in the decomposition
211 variables (RC, chemical element concentration) and parameters (k, m, B_o, B_1 and B_2). Because
212 the forest site had a significant effect, we used the analysis of covariance (ANCOVA) to
213 investigate the effects of microsite and litter type on the decomposition parameters and included
214 forest site as a covariate. Due to the unbalanced design, we first ran the analysis including only
215 the common microsite types (understorey of *Q. canariensis* and *Q. suber*) of the two forest sites,
216 and we then analysed the differences between the microhabitats within each site. Post hoc
217 comparisons were made using the Fisher LSD test. Type I error inflation resulting from repeated
218 tests was controlled using a false discovery rate procedure (FDR), as recommended by García
219 (2003).

To test for interactions between litter types i.e. non-additive effects of the species litter mixture on decomposition, we evaluated whether the categorical factor of individual vs. 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 to 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 (Fig. 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* (Fig. 1, Supplementary Fig. S1). However, both oak species converged to similar carbon values during the second year. We observed no interaction between species litter, i.e. 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, $p<0.474$) (Fig. 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 Fig. 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, i.e. 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 ($p<0.022$, Fig. 2). Similar but not

significant 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 Fig. 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 immobilisation and release over time differed among elements as revealed by the changes in their concentrations (Fig. 3, Supplementary Table S1) and amounts (Fig. 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 (Fig. 4) matched by a strong decrease in its concentration (Fig. 3, Supplementary Table S1). The concentrations of Zn and Mn showed monotonic

306 increases. The B_0 values for the two litter types differed significantly for all the chemical
307 elements studied, whereas differences in the parameters B_1 and B_2 were found for Ca, P, Mn and
308 Zn (Supplementary Table S1). The differences in element net loss between the litter types
309 indicated a higher and faster nutrient release (for Ca, Mg, P and S) from *Q. canariensis* litter
310 (Fig. 4). Nitrogen showed a distinctively different release pattern for the two oaks, being
311 relatively immobilised in *Q. suber* litter but released from *Q. canariensis* litter. Calcium was
312 immobilized during the first 6 months in *Q. canariensis* litter, but longer (12 months) in *Q. suber*
313 litter.

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

319 Predictors of litter decomposition

320 Both litter type and microsite environment affected decomposition parameters although the
321 relative magnitude of their effect (measured as the conditional R^2) differed and changed over
322 time. On average, microsite (as a categorical predictor) significantly explained a 4.4% of the
323 variance of the parameters related to early (3.4% of k and 5.3% of RC at 6 months) and a 4.5%
324 of the variance of the parameters related to late decomposition (3.7% of m and 5.2% of RC at 24
325 months). The variance explained by litter type decreased from early (35.2% of k and 28.4% of
326 RC at 6 months) to late (15.9% of m and not significant for RC at 24 months) decomposition
327 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 while 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 were related to lower decay rates but lower limit values (Fig. 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 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 change 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 (Fig 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 et al. (2000) proposed a three-phase decay model with an early decomposition stage, when the rapid decay of soluble and

373 non-lignified carbohydrates is regulated by N, P and S contents, a late decomposition stage,
374 when decay is regulated by the degradation of lignin, and a final or "humus-near" stage. The
375 turning point between early and late stages of decomposition is often encompassed by a peak in
376 Ca immobilization followed by a loss indicating the onset of net lignin degradation (Berg and
377 McClaugherty, 2008). Litter N has often being identified as a rate-enhancer factor for early
378 decomposition (Gallardo and Merino, 1993; Berg, 2000; Hobbie et al., 2012). The litter C:N
379 ratio, as an index of the nutritional balance, has also been found to affect microbial activity and
380 regulate the nutrient dynamics of the litter (Enríquez et al., 1993; Güsewell and Gessner, 2009).
381 However high initial litter N concentration also suppress lignin-degradation rates by hindering
382 the formation of lignolityc enzymes in the population of lignin degrading organism (white rot
383 fungi) thus impeding litter decomposition in the late stage (Eriksson et al., 1990; Hatakka, 2005).
384 Our study reveals that litter N can reverse its effect from rate-enhancer to rate-retarding in a two-
385 year period.

386 Previous studies have shown a strong and positive relationship between litter Ca and
387 decomposition rates in temperate forests (Chadwick et al., 1998; Hobbie et al., 2006). Calcium
388 supports the growth of white rot fungal species and is an essential cofactor of the lignin-
389 degrading enzymes of the decomposer microflora (Eriksson et al., 1990). The emergence of litter
390 Ca as a predictor of early decomposition together with the concentration and immobilization
391 patterns observed in this study suggests that degradation of lignin is already important in this
392 early stage of decomposition. Davey et al. (2007) reported an early onset of lignin degradation
393 on *Quercus robur* litter indicated by a significant correlation of decay rate and essential lignin
394 degrading co-factors such as Ca and Mn. Litter Ca has been related to increased microbial
395 activity, fungal and earthworm abundance and diversity and forest floor removal rates (Berg et

al., 2003; Reich et al., 2005; Hobbie et al., 2006; Aponte et al., 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 et al., 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 et al. (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 (i.e. *Q. canarienis* in this study, Fig. 3) would have a higher decay rate because of the lignolytic effect, but also 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 unexpected higher carbon loss from the perennial than the deciduous litter. There are contradicting evidences on the role of Mn during late decay stages. Berg et al. (2007) showed that the Mn concentration in the litter of five conifer species (range of 0.04 – 7.69 mg g^{-1}) affected positively 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 et al. (2007) found that litter Mn was not related to the limit value of decomposition of oak litter, but it was positively correlated to 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

418 relatively restricted concentration range (0.66 – 1.27 mg g^{-1}). We have shown that certain litter
419 nutrients, i.e. N, Ca and Mn, exert different effects on determining litter decomposition over
420 time, highlighting the importance of addressing all stages of decomposition when studying the
421 factors controlling carbon cycling and revealing that litter that initially decomposed faster might
422 as well generate the largest pool of accumulated carbon.

423

424 ## Tree species' environment effect on decomposition changes over time

425 Differential tree species environment significantly influenced decomposition although the
426 magnitude of this effect was smaller than the litter type effect and it mostly affected the
427 deciduous litter decay. The effect exerted by the tree species environment also reversed during
428 the decomposition process (like the litter type effect), but in this case the pattern was the
429 contrary. Decay beneath the deciduous oak, where soil was richer in nutrients, tended to be
430 slower during the early stage but to proceed further during the late stage. Soil N and P, and soil
431 moisture were the variables best related to litter decay. The role of soil nutrient availability on
432 litter decomposition processes is still poorly understood, while most studies focus on litter
433 nutrients (Davey et al., 2007; Strickland et al., 2009; Berg et al., 2010). Soil N was negatively
434 related to initial decay rate while it promoted an extended decomposition in the late stage. The
435 effect of exogenous N on litter decay has been studied in natural occurring gradients and
436 experimental conditions (e.g. McClaugherty et al., 1985; Hobbie, 2008; Hobbie et al., 2012) but
437 the observed effects have been inconsistent. Higher N availability sometimes increased initial
438 decay rates while most often had a negligible or even negative effect on decomposition
439 (Prescott, 1995; Hobbie and Vitousek, 2000). These studies suggest that soil N effect on decay

rates depends on the quality of the decomposing litter (McClaugherty et al., 1985; Hobbie and Vitousek, 2000; Hobbie et al., 2012). We can hypothesise 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, i.e. the deciduous leaf litter decomposed faster in environments other than its own. This interaction was contrary to the expected under the home-field advantage hypothesis (Vivanco and Austin, 2008; Ayres et al., 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 chonosequence 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 as key factors controlling soil microbial biomass (Aponte et al., 2010b). To this date few studies have investigated the influence of tree species on decomposition via the environmental conditions they generate (Hobbie et al., 2006). Our results suggest that the magnitude of tree species effect varies depending on the litter quality and soil conditions, thus inviting to further explore the circumstances that would magnify this effect.

Nutrient loss rates differed between litter types

462 Chemical elements differed in their litter decomposition dynamics although all the chemical
463 elements (except Mn and Cu) exhibited similar relative mobility in the two litter types. On
464 average, the elements were released in the order K>Mg>C>P>Mn>S>N>Ca>Cu>Zn (Figure 4).
465 Some patterns of litter nutrient release described here are similar to those from other temperate
466 forests: the rapid release of K is typically reported from a broad range of forest ecosystems
467 (Attiwill, 1968; Berg, 1986; Blair, 1988), and the increasing concentration and immobilization of
468 Zn has been related to throughfall input (Laskowski et al., 1995). In contrast, other elements
469 have shown a particular dynamics in this studied forest. For example, in other studies P is
470 immobilized at the initial stages of decomposition and subsequently released (Staaf and Berg,
471 1982; Maheswaran and Attiwill, 1987). However, this immobilization phase did not occur in this
472 experiment. Other studies showed continue loss of Ca, Mg and Mn, but the patterns reported here
473 were different. In general, distinct patterns in the dynamics of particular chemical elements in
474 various forest ecosystems reflect the different availabilities of nutrients to decomposers. Thus
475 those elements with concentrations below the limiting threshold for decomposers would be
476 immobilized in litter (Swift et al., 1979; Staaf and Berg, 1982). We have observed that N and Ca,
477 early rate-enhancer factors, were immobilised in the litter during the early decomposition stages
478 whereas Mn was immobilised during the late stages of decomposition. These temporal patterns
479 reflect the changes in the factors controlling decay as decomposition progresses, litter quality
480 changes and decomposer requirements vary.

481 An important contribution of this study into understanding tree species effect on
482 decomposition and ecosystem properties was to reveal that, despite the patterns of nutrient
483 concentration during the decomposition process were similar for both oak species, the patterns of
484 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 higher proportion that the litter of the perennial oak species thus inducing an elevated fertility beneath its canopy and a 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 et al., 1998b; Augusto et al., 2002; Aponte et al., 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, the carbon accumulation and the 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 higher fraction of slowly decomposable mass than the coexisting perennial oak (*Q. suber*), therefore producing a larger pool of accumulated stabilised carbon. This implies that initial litter decay rate and decomposition limit value might be uncoupled and thus litter that decompose 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 a highest initial nutrient concentrations and a 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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676 FIGURE LEGENDS

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

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

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

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

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

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TABLE LEGENDS

Table 1. Description of the soil beneath the oak trees where litterbags were incubated in the two studied forests (data taken from (Aponte et al., 2010b; Aponte et al., 2011) and unpublished results). Mean (St. dev.)

Table 2. Initial concentration (mean ± st. dev.) of chemical elements in decomposing leaf litter. Differences between oak species were tested with one-way ANOVA. Significant differences are indicated by bold-face P values

703 TABLES

704 **Table 1.**

		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)

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711 **Table 2.**

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

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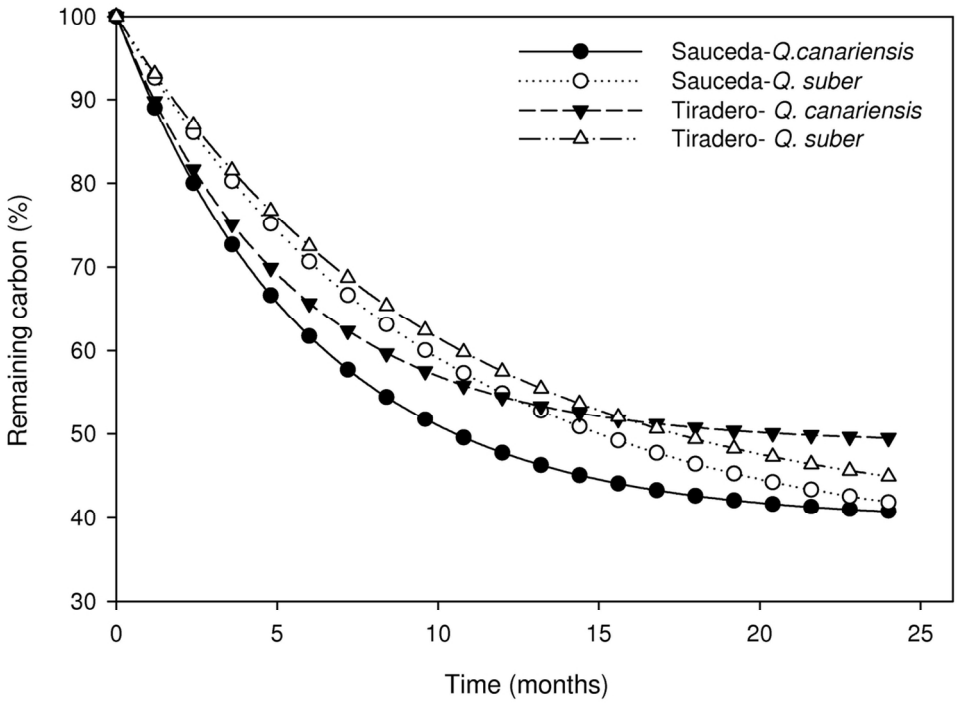

Fig. 1. Predicted variation in the remaining carbon (%) of leaf litter with time as a function of forest site and oak species, using the fitted asymptotic model.
118x91mm (300 x 300 DPI)

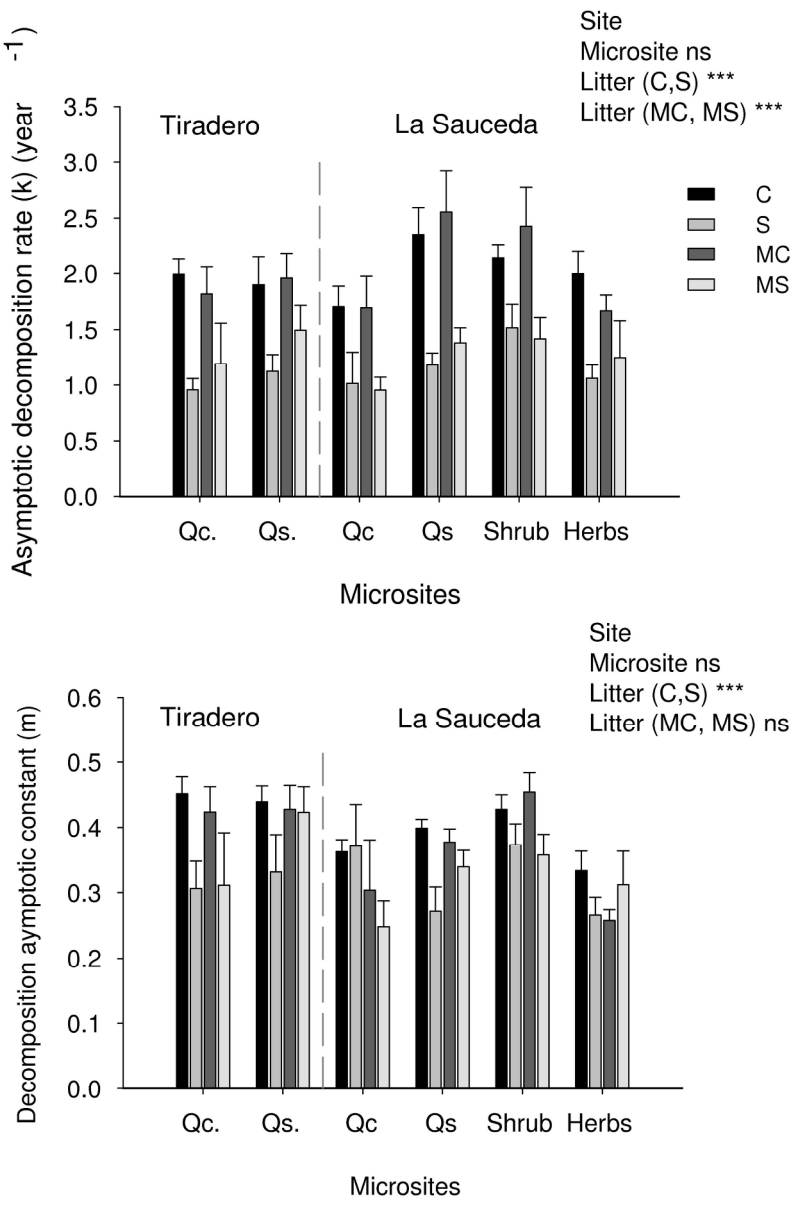

Fig. 2. Decomposition constants (mean+SE) of the single (C-Q. canariensis, S-Q. suber) and mixed (MC- Q. canariensis, MS- Q. suber) litters (*** p<0.001, ** p<0.01, *p<0.05, ns not significant).
188x272mm (300 x 300 DPI)

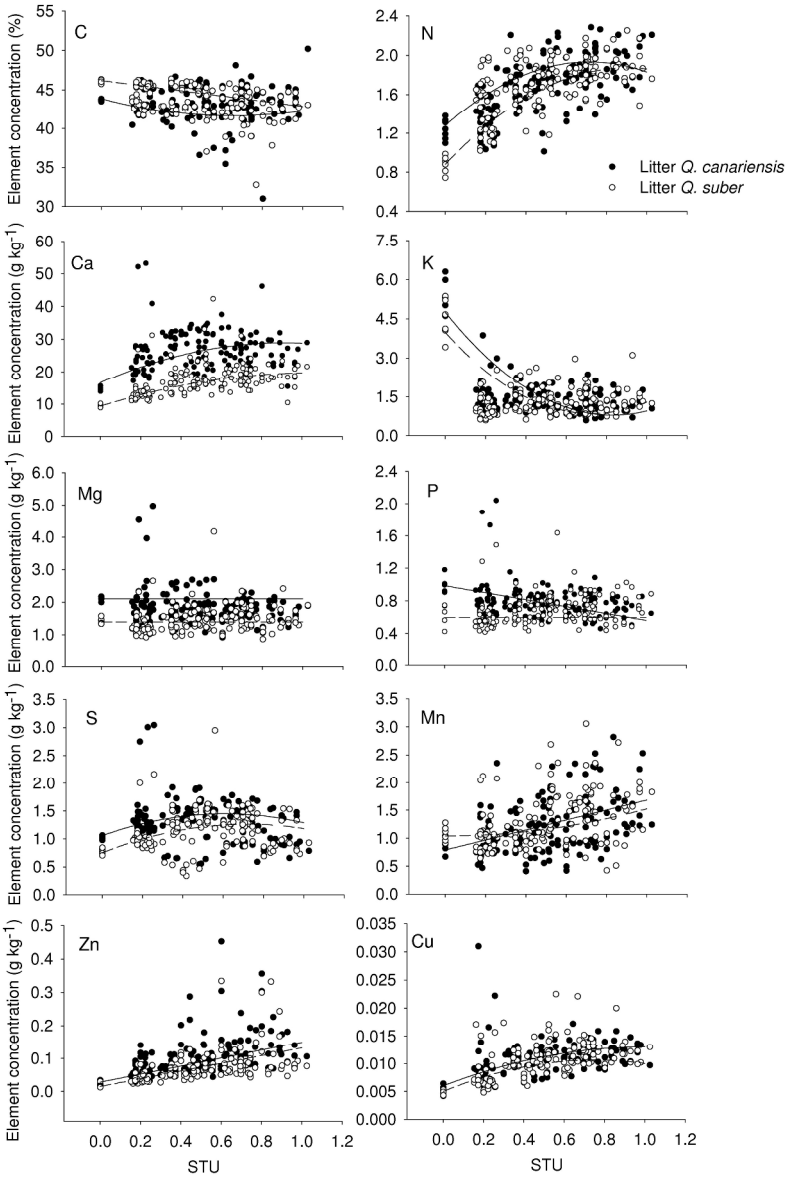

Fig. 3. Dynamics of the concentration of chemical elements in the decomposing leaf litter of Q. canariensis (solid line and filled circles) and Q. suber (dashed line and hollow circles). Error bars indicate 95% CI. Time is expressed in standardised time units (STU=time (yr) x decomposition constant k).
260x390mm (300 x 300 DPI)

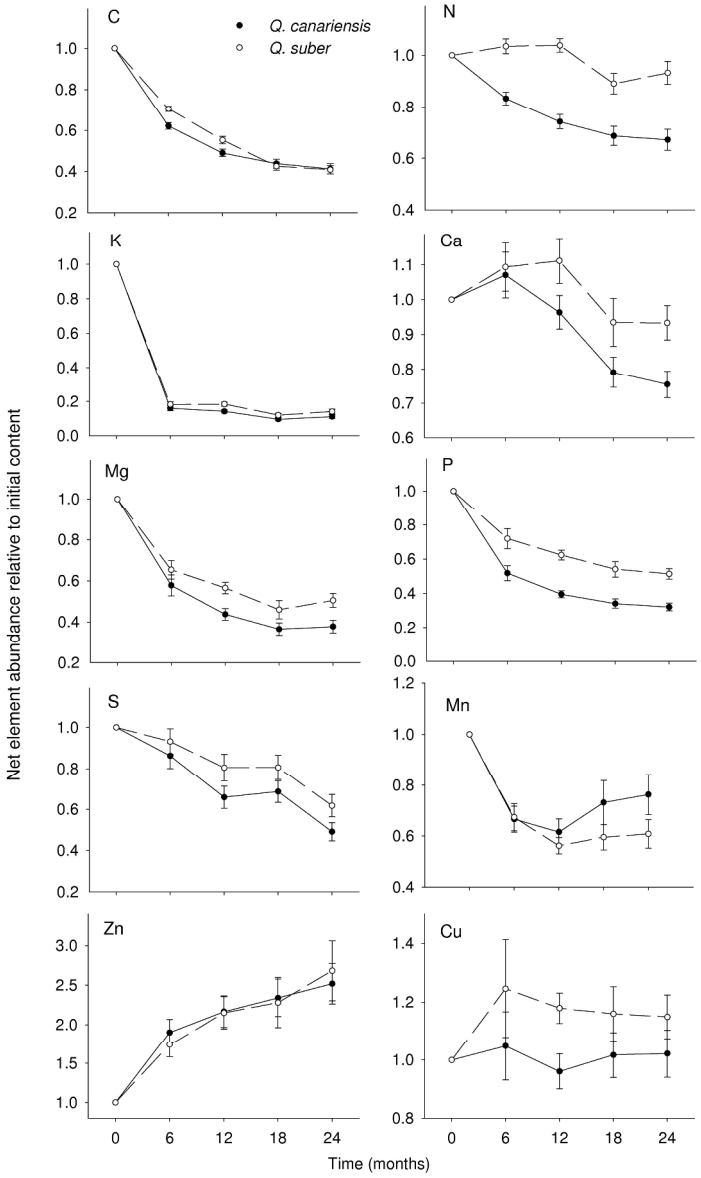

Fig. 4. Dynamics of the net immobilisation of elements in the decomposing litter of Q. canariensis (solid lines and filled circles) and Q. suber (dashed lines and hollow circles) during the 2 year experiment. Values are relative to initial element abundance.
267x443mm (300 x 300 DPI)

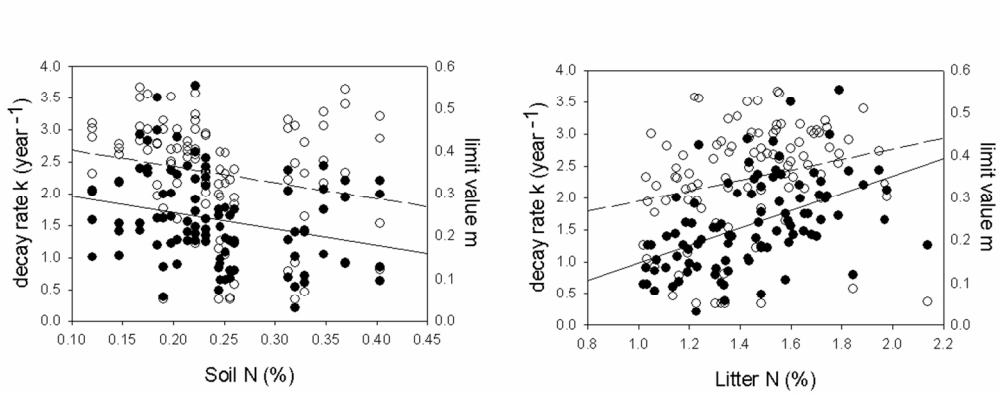

Fig. 5. Variation of the asymptotic decay rate (k, filled circles) and the limit value of the decomposition (m, hollow circles) in relation to the N concentration in the soil and litter of the studied oak trees. Increasing decay rate indicate a faster early decomposition while increasing limit value indicate a higher fraction of slowly decomposable litter.
195x77mm (150 x 150 DPI)

Supplementary material

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 *** $p<0.001$).

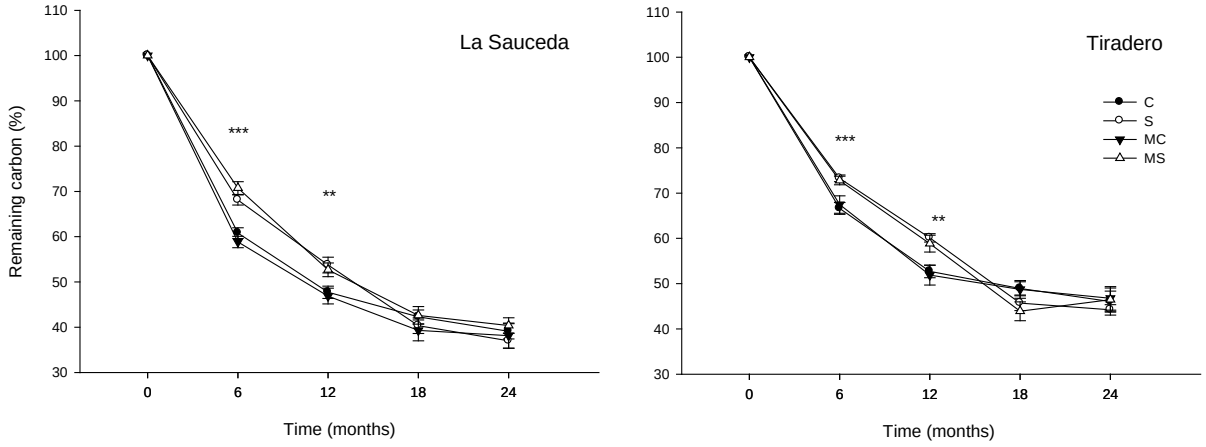

Figure S2. Remaining carbon (%) after 6 and 24 months for *Q. canariensis* and *Q. suber* leaf litter in the four microsites at Sauceda. One standard error of the mean is plotted.

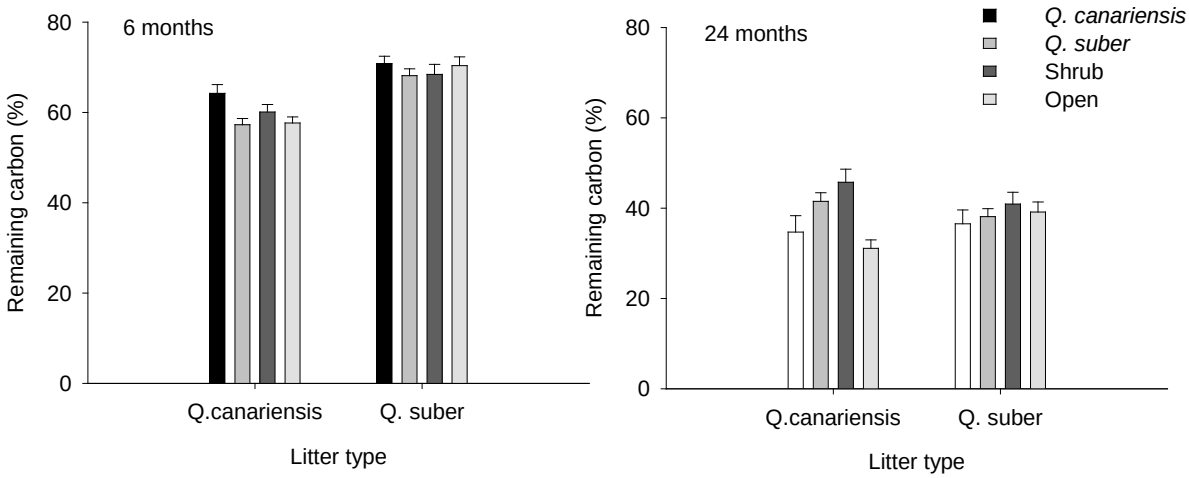

Figure S3. Average concentrations of chemical elements after the 2-year experiment for each site and microsite (*** p<0.001, ** p<0.01, *p<0.05, ns not significant).

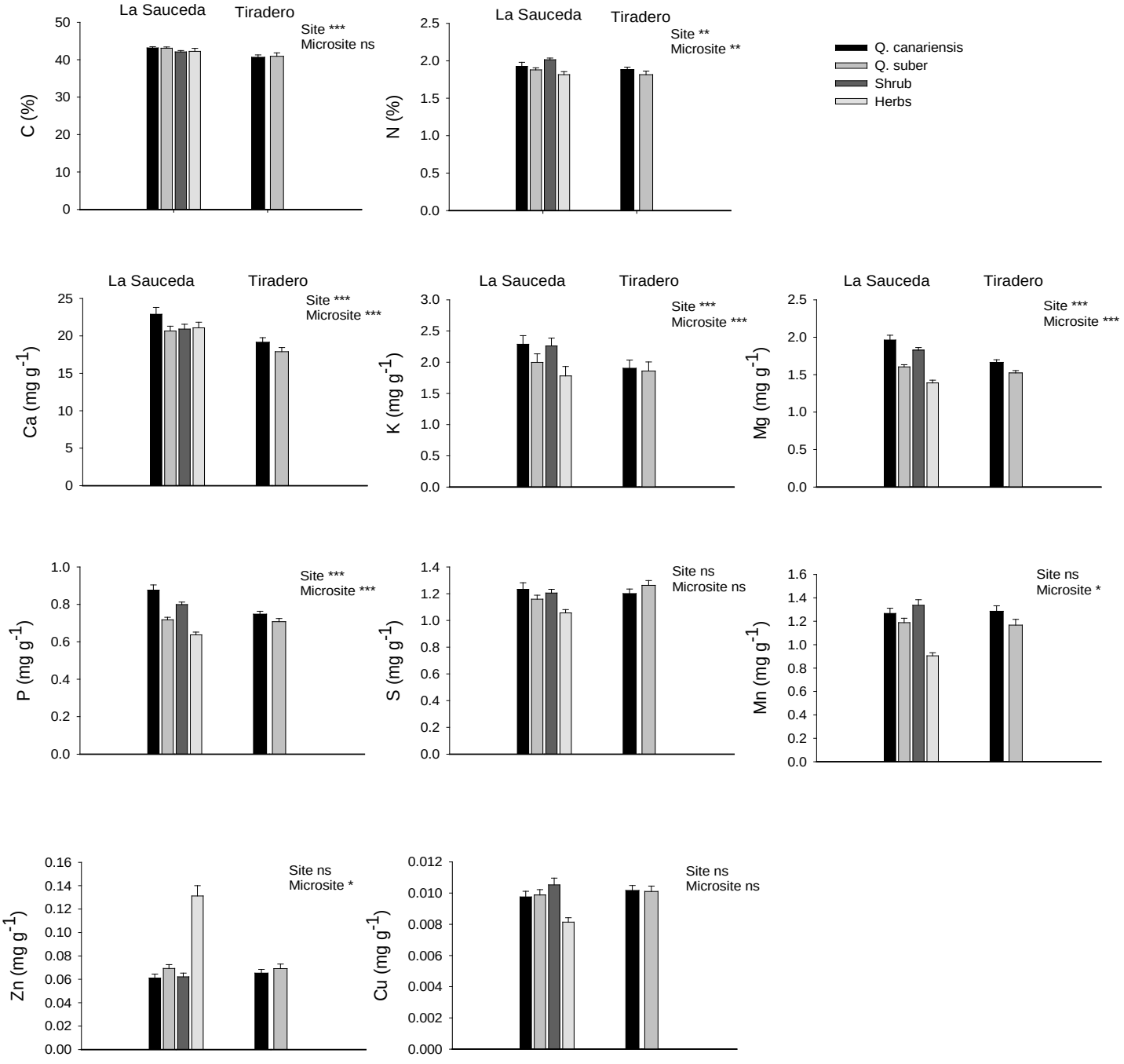

Table S1. Relation between element concentrations (Y) and standardised time by the decomposition constant (ST) for leaf litter of *Q. canariensis* (C) and *Q. suber* (S). Regression model: $Y=B_o+B_{1*}ST+B_{2*}ST^2$; the significance of the parameters is indicated (*** p<0.001, ** p<0.01, *p<0.5, ns=not significant). Superscript letters (a,b) indicate significant differences between litter types for each element and parameter (p<0.05).

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 ***

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). 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. unmeasured effects. Both significance of the categorical variables (p (χ^2)) and conditional variance retained are shown.

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	-