**RESEARCH ARTICLE**

# Dry weight loss in leaves of dominant species in a successional sequence of the Mesopotamian Espinal (Argentina)

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## Abstract

*Aim of study*: To compare litter decomposition dynamics among different species within a single forest type and also between a single species in different forest successional stages.

*Area of study*: Different forests of a known successional sequence of the Mesopotamian Espinal, placed in Villaguay Department, Entre Ríos Province, Argentina.

*Material and methods*: A standard "litter bags" technique was employed. Chemical analyses of C and N were performed for leaves. A regression analysis was applied and data were fitted to a double exponential model. Means estimated among forests and species within each forest were compared using the Tukey-Kramer test.

*Main results*: The model predicted that leaves would completely mineralize in the mid-term. Leaf decomposition rate in different species (both in the Secondary forest and Mature forest) had dry matter residues in the following decreasing order: *Acacia caven* > *Prosopis nigra* > *Prosopis affinis* > *Celtis ehrenbergiana*.

*Research highlights*: Successional stage was not found to be a factor determining the decomposition rate among species. Different decomposition rates, observed among different species, would not be attributed to initial quality of residues in terms of C and N, but would be associated with a positive feedback process related to nutrient cycle; thus, a greater decomposition would increase nutrient availability and, consequently, litterfall input.

**Additional keywords:** organic matter; decomposition; litter; dry forest; modeling; plant succession.

**Abbreviations used:** *IF* (Initial Forest); *MF* (Mature Forest); *SF* (Secondary Forest).

## Introduction

Leaf litter decomposition, as a process associated with biological activity of soil microorganisms, is related to microclimatic factors, such as soil temperature and moisture (Pérez-Harguindeguy *et al*., 2007), leaf litter quality (associated with chemical composition of the initial material; Carrera *et al*., 2009) and characteristics of invertebrate and microorganism communities (Torres *et al*., 2005; Vega Ávila *et al*., 2010). As a consequence, decomposition rate is a complex process that has specific characteristics for each ecosystem (Xu *et al*., 2010).

Decomposition is a biological process whereby litterfall inputs are converted into $CO_2$ and inorganic compounds. Decomposition of organic residues is a key process in internal nutrient cycling, with a positive return to forest productivity (Gallardo & Merino, 2007).

While degrading processes are associated with leaf aging itself (even when the leaf is still on the tree), the most frequently used method to determine the rate of leaf decomposition has been the used of litter bags, which determines the loss of leaf dry weight (Aceñolaza & Gallardo, 1994; Rovira & Rovira, 2010; Carranza *et al.,* 2012; Bueis *et al.,* 2017). The pattern of leaf litter dry weight loss generally comprises two phases: an

initial phase, characterized by rapid leaching of soluble compounds and decomposition of labile substances such as sugars, phenols, starches, and proteins; and a second, much slower phase, involving the initiation of degradation of recalcitrant substances such as cellulose, hemicellulose, tannins and lignin (Arellano *et al*., 2004).

Decomposition of leaf litter deposited on the floor of natural forests or of forests subjected to silvopastoral use implies the incorporation of nutrients to the soil that is relevant for sustainability of those ecosystems (Prause *et al*., 2012). Decomposition of leaf litter inputs makes nutrients available to the plant community (Carranza *et al*., 2012). Nutrient flux can be slowed down, with key chemical elements for forest productivity being transiently retained in some of the different compartments (aerial biomass, litter and soil organic carbon; Gallardo & González, 2004).

The Argentine Espinal is a forest ecosystem located between 28º and 40º S, to the south of the Chaco Ecoregion, with an estimated total area of 33,000,000 ha (Lewis *et al*., 2006); it forms a large arch that surrounds the *Pampas* grassland to the west. This ecosystem is dominant in an extensive area of the South American Southern Cone and is subjected to silvopastoraluse.

The Mesopotamian Espinal (Entre Ríos, Argentina) includes the forests of Espinal Phytogeogaphic Province, Ñandubay District (Cabrera, 1976). In several areas of Entre Ríos, an intensive modification of the Mesopotamian Espinal is observed in the rural landscape due to the advance of the agricultural frontier, which has produced an important reduction of the forest area (Arturi, 2006) accompanied with an increasing heterogeneity pattern (Aceñolaza, 2000). As a consequence, primary forests have been transformed, generating a successional sequence characterized by secondary forests that are degraded by forest exploitation for timber production (focused on the logging of *Prosopis nigra*), subsequent inappropriate livestock management, or as a consequence of excessive firewood extraction (affecting the species *Acacia caven* and *Prosopis affinis*).

After deforestation and abandonment of croplands, an initial successional stage (*IF*) is established, dominated by a woodland of *A. caven*; subsequently, this secondary formation (*SF*) is colonized by *P. affinis*, which may be defined as an intermediate stage; finally, the mature forest stage (*MF*) is reached, with the appearance and dominance of *P. nigra*, thereby the primary forest is reestablished (Mendoza *et al*., 2012).

To determine the sustainability of silvopastoral systems in the Mesopotamian Espinal, it is important to characterize the dynamics of leaf decomposition of the tree typical species that compose the successional sequence of these forests, since such decomposition regulates nutrient return to the soil and, therefore, largely conditions its fertility.

The aims of this work were to: a) determine the process of weight loss via leaf decomposition, by fitting a regression model; b) determine if a single species has different decomposition dynamics according to the forest successional stage; and c) compare decomposition dynamics among different species within a single forest type.

## Material and methods

### Study area

The study area is in the province of Entre Ríos, Argentina, between 31º 47' 59'' S and 59º 11' 38'' W. Within this area, three experimental plots were selected and studied, each belonging to one of the stages of the successional sequence (Fig. 1): *IF*, *SF* and *MF*. *IF* is a monospecific forest of *Acacia caven* (Molina) Molina; this area had been a cropland that was abandoned in 1998 and later naturally colonized by this species. *SF* is dominated by *Prosopis affinis* Spreng, and presents other typical species, such as *A. caven* and *Celtis ehrenbergiana* (Klotzsch) Liebm.; some of the trees are up to 50 years old. *MF* is the reestablished primary forest dominated by *P. nigra* (Griseb.) Hieron., with the presence of *A. caven*, *P. affinis*, and *C. ehrenbergiana*; some trees of the dominant species are more than 80 years old.

These three forest formations presented a production of litter of 1.14, 2.95, and 2.91 Mg DM/(ha·yr), (DM: dry matter) respectively; they have been evaluated by Mendoza *et al.* (2012) who showed the real heterogeneity of the landscape, with a floristic richness represented by species of the family *Fabaceae* (*A. caven*, *P. affinis*, and *P. nigra*) that possess biological binding capacity of the atmospheric $N_2$; they are potentially important in terms of the transfer of N to the soil in processes of decomposition, and only *C. ehrenbergiana* (without the ability to biologically fix the atmospheric $N_2$) belonging to the family *Celtidaceae*, would not have this potentiality (Mendoza *et al*., 2014).

### Characterization of climate, soil of the study area

The climate in the study area is temperate-humid; mean annual precipitation is above 1000 mm/yr, with important interannual variation (INTA, 2000). Rainfall occurs throughout the year, with notable increases in autumn and spring. Mean annual temperature is 16 ºC,
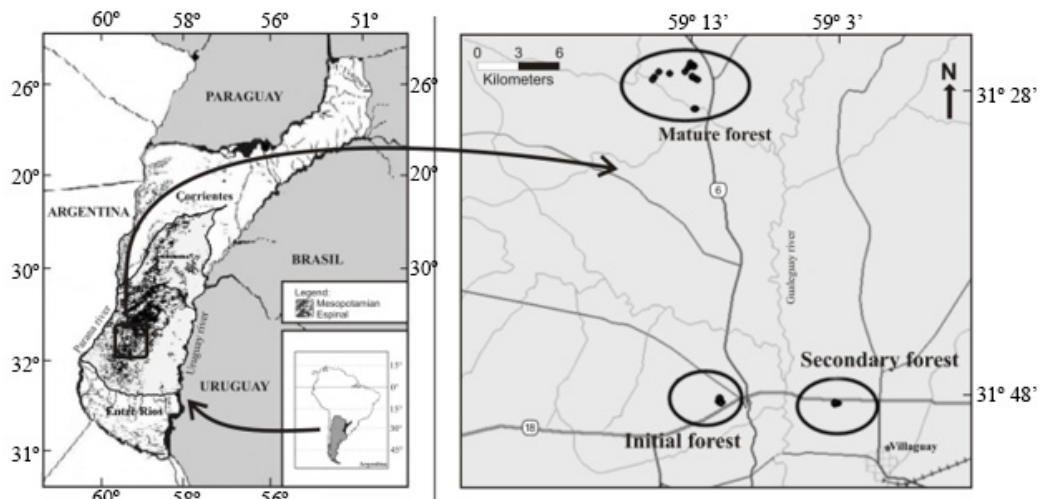
**Figure 1.** Location of the experimental plots of studied forests: initial forest dominated by *Acacia caven*; secondary forest dominated by *Prosopis affinis*; and mature forest dominated by *Prosopis nigra* (Entre Ríos province, Argentina).

with the mean of the coldest and hottest months being 11 °C (July) and 25 °C (January), respectively.

Climatic data shown in Fig. 2 correspond to the period when the decomposition assay was performed. Those data points were obtained from the automatic meteorological station of the *Bolsa de Cereales*, located near the study area (http://centrales.bolsacer.org.ar/pluviometros/).

Soils were formed during the Quaternary from the deposition of sediments corresponding to Hernandarias Formation (Aceñolaza, 2007). Those materials were of palustrine-lacustrine origin and originated soils with high contents of expanding clays (montmorillonite). These soils are, therefore, of clay texture, deep, with poor drainage, with calcareous concretions from -90 cm in depth downwards, and scarce in available P. Taxonomically, soils in the area correspond to the order *Vertisoil*, with slightly acid pH (6.3 ±0.7) and, in general, with high soil organic matter (SOM) content of 5.4 ±3.0%, depending on land use intensity (INTA, 2000).

**Method used for the study of dry weight loss from leaf decomposition**

The study was conducted using the litterbags standard technique (Aceñolaza &Gallardo,1994;Carranza *et al*., 2012). The bags were 18 x 15 cm² in size, and made of 100-μm mesh; this mesh size was selected because the tested species have very small follicles.

Leaves were collected in autumn (May 2010) from the crown of four trees per species and forest type, using pruning shears as proposed by León *et al*. (2009). The leaves were dried in an 80 °C heater (Dalvo Sb343, Argentine) until constant weight was obtained. Then, 3.00 g of leaf of each species were weighed and placed in litter bags. The leaves of the different species were not mixed; the experimental design consisted by placing litterbags in the different forests, as follows: IF (32 litter bags of *A. caven*), SF (32 litter bags of *A. caven*, 32 of *P. affinis*, and 32 of *C. ehrenbergiana*), and in *MF* (32 litter bags of *A. caven*, 32 of *P. affinis*, 32 of
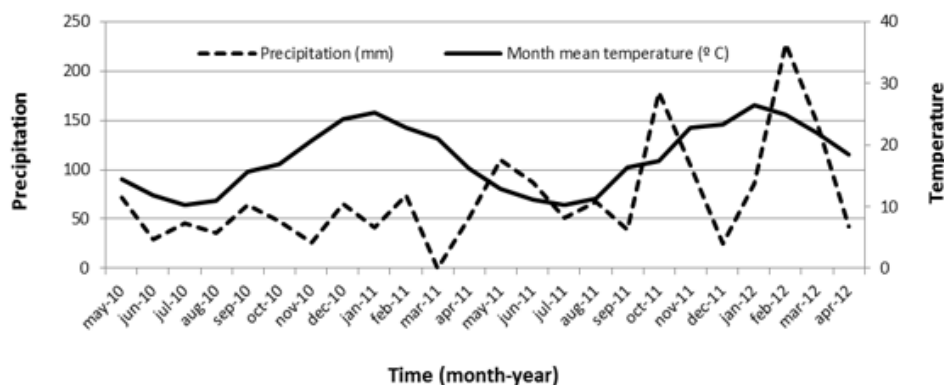


**Figure 2.** Evolution of mean temperature and monthly precipitation for the period when the decomposition assay was performed. Villaguay Department (Entre Ríos, Argentina).

*C. ehrenbergiana*, and 32 of *P. nigra*). Litter bags were placed on the floor under canopies of trees, according the species studied in the successional sequence, simulating conditions of natural decomposition. Later, litterbags were randomly removed (four bags per forest and per species) between May 2010 and May 2012, at: 7, 20, 40, 80, 160, 300, 500, and 700 days since the start of the assay. The leaf litter was taken to the laboratory, dried in an 80°C heater until constant weight was reached, and weighed with an electronic balance (T-Scale, NHB-600, Taiwan).

**Chemical analyses of leaves**

Chemical analyses of C and N were performed, and the C/N ratio was calculated for leaves, per species and forest, taken from samples of leaf litter fell during the 2009-2010 period. A sample from each season, year and species was analyzed.

All samples were dried in an 80 °C heater and then ground with a ball mill (Retsch, MM301, Germany).

Chemical analyses were then performed to determine C content (total Carbon, mg C/g dry matter) using dry combustion (Wösthoff, Carmhograph12, Germany), and N content (total nitrogen, mg N/gDM) using a segmented flow auto-analyzer (Bran+LuebbeAA3, Germany).

**Statistical analyses**

A regression analysis was applied, considering residual dry matter as dependent variable and time as independent variable (Jenny *et al.,* 1949; Olson, 1963; Rovira & Rovira, 2010).

Data were fitted to a double exponential model (Bunnel & Tait, 1974) plus a constant, following the Eq. [1]:

$$X_t/X_0 = ae^{-(k_1 t)} + be^{-(k_2 t)} + c \quad [1]$$

where $X_0$= initial amount of leaf litter (g DM); $X_t$= amount of remaining material (g) after a time period $t$; $a$= mass of the fraction of fast decomposition rate in (g/g); $b$ = mass of the fraction of slow decomposition rate (g/g); $c$ = asymptote that quantified the recalcitrant fraction; $k_1$= decomposition rate of the fast fraction (yr$^{-1}$); $k_2$= decomposition rate of the slow fraction (yr$^{-1}$); $t$= time (yr) spanned between $X_0$ and $X_t$. The restriction $a + b + c = 1.0$ was also considered.

With the parameters ($\hat{a}, \hat{b}, \hat{c}, \hat{k}_1, and \hat{k}_2$) obtained in each of the non-linear regressions, the values of dry matter weight loss ($X_t/X_0$; in g DM/g) for the 8-time periods used in the model (7, 20, 40, 80, 160, 300, 500, and 700 days) were estimated.

For each of the parameters calculated, an analysis of variance was performed with two inter-individual factors, using a hierarchical design (forest and species nested within forest) using the Eq. [2]:

$$y_{jk(i)} = \mu + \alpha_i + \beta_{j(i)} + \varepsilon_{jk(i)} \quad [2]$$

where $y_{jk(i)}$ = value estimated for parameter $y$ in the sample $k$ of the species $j$ in the forest $i$; $\mu$ = general mean effect; $\alpha_i$ = principal effect of forest $i$; $\beta_{j(i)}$ = principal effect of species $j$ within forest $i$; $\varepsilon_{jk(i)}$ = random error for the value estimated of $y$ in sample $k$ of species $j$ within forest $i$, with $\varepsilon_{jk(i)}$ $N(0, \sigma^2)$, where $\sigma^2$ is random variance of the model.

The different values of $i$, $j$ and $k$ were: $i = 1,2,3$ for initial, secondary and mature forests, respectively; $j = 1$ (species *A. caven*) if $i = 1$; $j = 1,2,3$ for the species *A. caven*, *P. affinis* and *C. ehrenbergiana,* respectively, if $i = 2$; and $j = 1,2,3,4$ for the species *A. caven*, *P. affinis*, *C. ehrenbergiana* and *P. nigra,* respectively, if $i = 3$; and $k = 1,2,3,4$ for the replications of each species within each forest.

Finally, the means estimated among forests and species within each forest were compared using the Tukey-Kramer test with a significance level of 0.05. Normality of residuals was compared using the Kolmogorov-Smirnov test, and homogeneity of variances, via the Levene test, using SAS 9.2 statistical package (SAS Inst., 2005).

The values corresponding to dry matter for each collection time (0, 7, 20, 40, 80, 160, 300, 500, and 700 days) were compared using a mixed linear model analysis of variance (McCulloch & Searle,2001) with two inter-subject factors employing a hierarchical design (forest and species nested within forest).

Mean concentrations of C, N and C/N were compared using a mixed linear model analysis of variance (McCulloch & Searle, 2001), considering two inter-subject factors employing a hierarchical design (forest and species nested within forest).

## Results

**Analysis of the loss of dry weight in leaves of the tree species of the Mesopotamian Espinal: fit of the model**

The results showed high values of coefficients of determination ($r^2$) in the fit of Eq. [1] (Table 1), which ranged between 95.1% and 99.3%. This model predicts that the leaves of all the species evaluated will be completely mineralized in the mid-term (a few years), since parameter $c$ (which would correspond to recalcitrant material with a too long decomposition time)

tended statistically to zero in the studied species (Table 1), whereas parameters $a$, $b$, $k_1$ and $k_2$ were statistically different from zero.

## Temporal patterns of leaf dry weight loss in species present in different forests

The comparison of the parameters obtained in the decomposition model, for a single species in different forests, showed that for *A. caven* (species present in the three forests of the successional sequence) parameter $a$ reaches an average value of 16% of the initial weight, without significant differences among forests. Decomposition constant ($k_1$) of fraction $L$ did show significant differences, with significantly higher values in *SF* (94.6/yr) than in *IF* and *MF*, which had values of about 50/yr (without significant differences between *IF* and *MF*; Table 1). This $L$ fraction of rapid decomposition had half-life times (ln2/$k_1$) for *A. caven* of 2.7, 4.9, and 5.3 days, for *SF*, *IF*, and *MF*, respectively, indicating that half of this fraction was lost in less than a week. Fraction $b$ did not show significant differences among forests, with $R$ being about 84% of the initial amount (and, therefore, the dominant fraction); decomposition constant ($k_2$) of $R$ did not show significant differences among forests for this species, with values close to 0.70/yr; and the calculated half-life time of this fraction (ln2/$k_2$) was approximately one year (Table 1).

Regarding *P. affinis* (a species common to both *SF* and *MF* forests), the comparison of coefficient $a$ did not show significant differences between forests, with $L$ fraction representing about one third in both forests. Decomposition constant $k_1$ of $L$ did not show significant differences between forests, with values close to 30/yr (also corresponding to

a half-life of about 8.4 days). Coefficient $b$ did not also show significant differences between forests, with fraction $R$ being close to 68%; similarly, constant $k_2$ did not show significant differences between *SF* and *MF*, with values close to 0.80/yr (Table 1).

Parameters $a$, $b$, $k_1$, and $k_2$ corresponding to *C. ehrenbergiana* (species common in *SF* and *MF*) did not also show significant differences between forests, as observed for *P. affinis* (Table 1).

Figures 3, 4a and 4b, show the temporal evolution of weight loss in the three forests for the species *A. caven, P. affinis* and *C. ehrenbergiana*, respectively.

*Acacia caven* (present in *IF*, *SF* and *MF*) and *P. affinis* (present in *SF* and *MF*) showed no significant differences in residual dry weight values (which were 22 and 15%, respectively; Figs. 3 and 4a) after 700 days of decomposition. Likewise, they showed similar temporal evolution of weight loss between those forests, since there were no significant differences between them in any of the compared sampling dates.

In contrast, *C. ehrenbergiana* showed significant differences in residual dry weight values at the end of the assay, with 9 and 11% in *SF* and *MF*, respectively (Fig. 4b); their temporal evolutions were similar up to 80 days of decomposition; after that sampling date, the recorded weight loss values were significantly higher in *SF* than in *MF* (Fig. 4b).

## Temporal patterns of leaf weight loss of different species within each type of forest

The comparison of parameters among species within a single forest showed significant differences both in

**Table 1.** Mean values (including ± standard error) of the parameters obtained by applying the double exponential model (Equation 1) and results of ANOVA by species and forest.

| Forest | Species | n | $A$ (g/g) | $b$ (g/g) | $c$ (g/g) | $k_1$ (y$^{-1}$) | $k_2$ (y$^{-1}$) | $r^2$ (%) |
|--------|---------|---|-----------|-----------|-----------|------------------|------------------|-----------|
| *IF* | *A. caven* | 4 | 0.15 (±0.06) a | 0.85 (±0.06) a | 0 | 52.2 (±15.5) b | 0.68 (±0.08) a | 95.1-97.8 |
| *SF* | *A. caven* | 4 | 0.18 (±0.06) Ba | 0.82 (±0.06) Aa | 0 | 94.6 (±15.5) Aa | 0.68 (±0.08) Ba | 96.3-97.1 |
| | *P. affinis* | 4 | 0.30 (±0.06) Aa | 0.70 (±0.06) ABa | 0 | 32.1 (±15.5) Ba | 0.82 (±0.08) Ba | 98.3-99.1 |
| | *C. ehrenbergiana* | 4 | 0.40 (±0.06) Aa | 0.60 (±0.06) Ba | 0 | 11.8 (±15.5) Ba | 1.03 (±0.08) Aa | 97.2-99.3 |
| *MF* | *A. caven* | 4 | 0.15 (±0.06) Ba | 0.85 (±0.06) Aa | 0 | 48.1 (±15.5) Ab | 0.70 (±0.08) Ba | 95.1-97.4 |
| | *P. affinis* | 4 | 0.33 (±0.06) Aa | 0.67 (±0.06) Ba | 0 | 24.5 (±15.5) Aa | 0.75 (±0.08) Ba | 96.5-98.1 |
| | *C. ehrenbergiana* | 4 | 0.28 (±0.06) Aa | 0.72 (±0.06) Ba | 0 | 32.9 (±15.5) Aa | 0.98 (±0.08) Aa | 96.3-97.5 |
| | *P. nigra* | 4 | 0.29 (±0.06) A | 0.71 (±0.06) B | 0 | 19.0 (±15.5) A | 0.73 (±0.08) B | 95.6-98.5 |
| Effect | "forest" | | *** | *** | ns | ** | *** | |
| | "species (forest)" | | *** | *** | ns | *** | *** | |

*IF*: initial forest, *SF*: secondary forest, and MF: mature forest. Different uppercase letters indicate statistically significant differences among different species within a single forest type, and different lowercase letters indicate statistically significant differences between shared species in different forests (Tukey-Kramer test, p<0.05). Significance levels for the effects "forest" and "species (forest)": ***($p$ <0.001); **($p$ <0.01); *($p$ <0.05) and ns "non-significant".
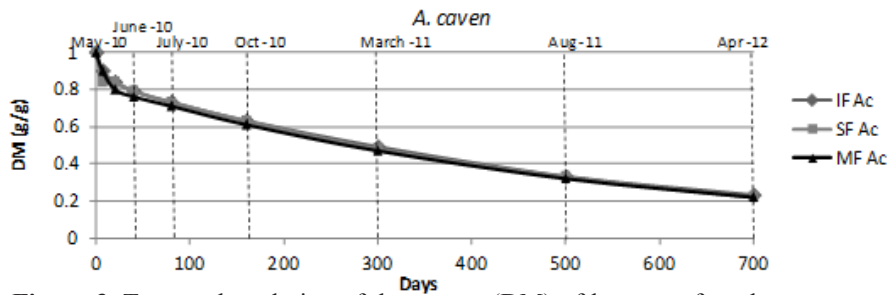
**Figure 3.** Temporal evolution of dry matter (DM) of leaves, referred as a proportion of 1.00 g DM of *A. caven* (Ac) in initial forest (*IF*), secondary forest (*SF*), and mature forest (*MF*). No significant differences (*p*<0.05) between forests for each date were found.
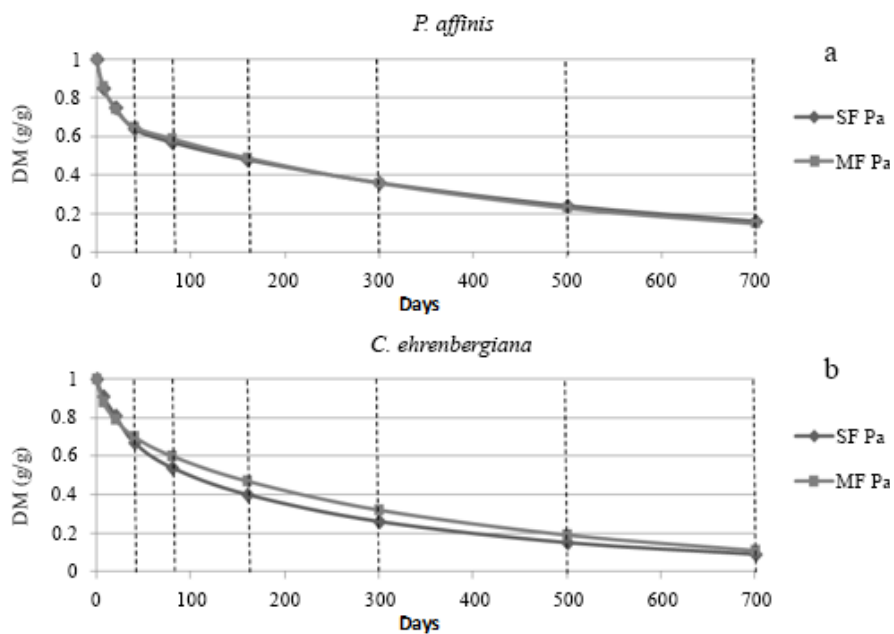


**Figure 4.** Temporal evolution of dry matter (DM) of leaves, referred as a proportion of 1.00 g DM of *Prosopis affinis* (a) and *C. ehrenbergiana* (b) in secondary forest (*SF*) and mature forest (*MF*). No significant differences (*p*<0.05) between forests for each date were found.

*SF* and *MF* (Table 1). Leaves of *C. ehrenbergiana* showed a significantly higher decomposition constant ($k_2$) of *R* fraction in both forests (*SF* and *MF*) than the constants of the three-leguminous species. Furthermore, leaves of *A. caven* in *SF* had significantly different values for parameters of *L* fraction (lower in *a* and higher values $k_1$) from those of the other two species present; in *MF* the same significant differences were observed for parameter *a*, but not for $k_1$.

Table 2 shows the leaf contents of C, N and the C/N ratio. The high values recorded in the leguminous species agree with the expected values.

Figures 5 and 6 show the temporal evolution of leaf dry matter, expressed as a proportion, for *A. caven, P. affinis, C. ehrenbergiana*, and *P. nigra* in secondary

(*SF*) and mature (*MF*) forests, respectively. Rates of weight loss were compared only among different species of *SF* and *MF*, since only one species was present in *IF*. In general, percentage of remaining dry matter at the end of the assay in the species of the Mesopotamian Espinal ranged between 22% in *A. caven* and 10% in *C. ehrenbergiana* (Figs. 5 and 6).

In *SF*, significant differences in temporal evolution of the three species were observed. As shown in Fig. 5, a higher loss of labile substances was recorded at the start of the decomposition process (7, 20, and 40 days) in *P. affinis* and *C. ehrenbergiana* than in *A. caven*; after 80 days and until the end of the assay, the three species differed significantly, with *A. caven* and *C. ehrenbergiana* being the species with the lowest and highest decomposition rates, respectively.

**Table 2**. Contents of C and N, and C/N ratio of leaves at the start of the decomposition assay (standard error is indicated in parentheses).

| Forest | Species | n | C | N | C/N |
|---|---|---|---|---|---|
| | | | (mg/g dry matter) | | |
| *IF* | *A. caven* | 8 | 495 (±21) | 24.5 (±3.5) | 20.2 (3.2) |
| *SF* | *A. caven* | 8 | 481 (±32) | 27.2 (±3.1) | 18.8 (4.5) |
| | *P. affinis* | 8 | 492 (±26) | 29.2 (±3.2) | 18.0 (3.9) |
| | *C. ehrenbergiana* | 8 | 486 (±45) | 24.8 (±7.0) | 19.6 (2.9) |
| *MF* | *A. caven* | 8 | 489 (±38) | 26.9 (±5.2) | 18.2 (5.5) |
| | *P. affinis* | 8 | 496 (±42) | 28.4 (±3.4) | 17.5 (4.9) |
| | *C. ehrenbergiana* | 8 | 480 (±56) | 26.2 (±5.8) | 18.3 (3.8) |
| | *P. nigra* | 8 | 497 (±51) | 28.3 (±5.4) | 18.3 (4.2) |
| Effect | "forest" | | ns | ns | ns |
| | "species(forest)" | | ns | ns | ns |

*IF*: initial forest, *SF*: secondary forest, and *MF*: mature forest. Forests were compared using the Tukey-Kramer test ($p<0.05$) for the effects "forest" and "species (forest)"; ns: non-significant.

## Secondary forest



**Figure 5.** Temporal evolution of dry matter (DM) of leaves, referred as a proportion of 1.00 g DM of *Acacia caven* (Ac), *Prosopis affinis* (Pa), and *Celtis ehrenbergiana* (Ce) in the secondary forest (*SF*). Different lowercase letters indicate significant differences ($p<0.05$) between species for each date.
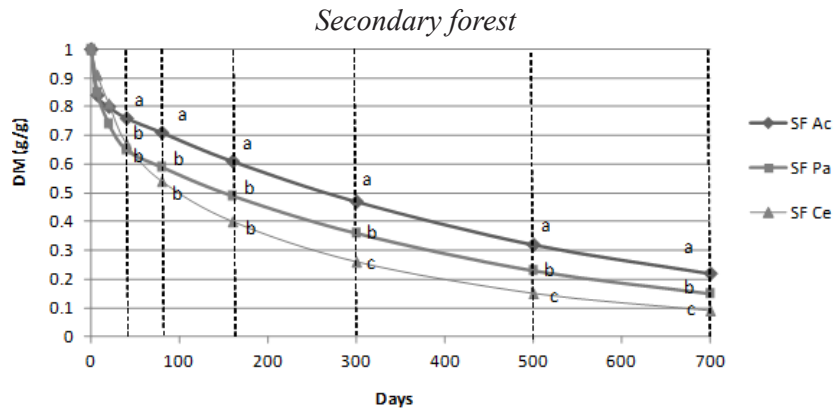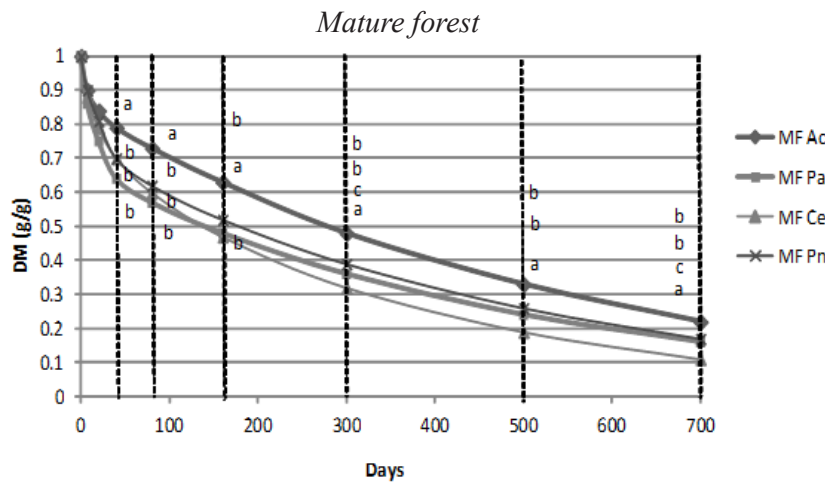
## Mature forest



**Figure 6.** Temporal evolution of dry matter (DM) of leaves, referred as a proportion of 1.00 g DM of *Acacia caven* (Ac), *Prosopis affinis* (Pa), *Celtis ehrenbergiana* (Ce), and *Prosopis nigra* (Pn) in the mature forest (*MF*). Different lowercase letters indicate significant differences ($p<0.05$) between species for each date.

Decomposition rate in *MF* did not show significant differences among the four species (*A. caven, P. affinis, C. ehrenbergiana*, and *P. nigra*) at the start of the assay (7 days). Between 40 and 160 days, however, *A. caven* differed significantly from the other three species. In addition, *C. ehrenbergiana* and *P. nigra* did not show significant differences between them (Fig. 6).

From day 300 and up to the end of the assay, the species with the lowest and highest decomposition rates were *A. caven* and *C. ehrenbergiana*, respectively, with significant differences from each other and from the other two *Prosopis* species (*P. affinis* and *P. nigra*). The last two species exhibited intermediate decomposition dynamics, with no significant differences between them on the different sampling dates of this study (Fig. 6).

## Discussion

### Dry weight loss in leaves of the tree species from the Mesopotamian Espinal

The model applied here (Bunnel & Tait, 1974) differs from those typically proposed in the specialized literature (Jenny *et al*., 1949; Olson, 1963; Aceñolaza & Gallardo, 1994; León *et al*., 2011) as it present the parameter *c* (corresponding to a highly recalcitrant fraction). This parameter predicts that leaves, of all the evaluated species, will mineralize in the short- to mid-term, since all the species yielded values close to zero (Table 1). While term *a* would correspond to the very labile fraction (*L*) that would decompose according to constant $k_1$, term *b* corresponds to the relatively resistant material (*R*) that decomposes according to constant $k_2$.

Total leaf decomposition might explain, among other aspects, the productive sustainability of these forests given by the effects of nutrient return via leaf litter.

Previous studies conducted by Mendoza *et al*. (2014) in the same study area showed that N and P are abundant elements in litterfall of these species; hence, an efficient recycling would allow soil fertility to be maintained (or even increased).

The rapid and complete decomposition in the Mesopotamian Espinal can be attributed to the low C/N ratio in leaves at the start of the assay, without significant differences among species (Table 2) as well as to the good soil moisture levels and optimum temperature values suitable for the development of microorganisms almost throughout the annual cycle (Rojas & Saluso, 1987). According to Aceñolaza & Gallardo (1994) and León *et al*. (2011), low C/N is a factor associated with a high leaf litter decomposition rate, besides the favorable climatic conditions of moderate temperatures and a short period of water deficit, which are characteristic in our study area.

Our results are somewhat different from findings of Carranza *et al*. (2012) for the Argentine Arid *Chaco* (involving different species), who recorded lower decomposition constants. The different behaviors between the Arid *Chaco* and the Mesopotamian Espinal may be attributed not only to the different C/N ratios but also to the presence of a marked annual period of water deficit in the Arid Chaco, which influences decomposition rate of the residual organic matter (Fioretto *et al.,* 2005). As these forests are not fertilized to promote herbaceous species production, it is important to conserve the diversity and density of N-fixing woody native species to ensure productivity of the silvopastoral system; Carranza *et al*. (2012) found that the presence of trees in selective clearings promotes nitrogen release from native pastures.

The comparison of the parameters among species within a single forest type (Table 1) showed that *A. caven* had the lowest coefficient *a* values, corresponding to the *L* fraction, with significant differences from the remaining species (both in *SF* and in *MF*), indicating a higher number of recalcitrant substances in *A. caven* leaves than in leaves of the other species. Therefore, the leaves of *P. affinis*, *C. ehrenbergiana*, and *P. nigra* may lose greater labile substances through leaching (water-soluble substances) than in *A. caven*; Aceñolaza & Gallardo (1994) and Prause *et al*. (2002, 2012) confirmed the occurrence of rapid labile and water-soluble substances leaching in species of subtropical forests used for silvopastoral activities.

By contrast, *A. caven* had the highest term *b,* which corresponds to fraction *R,* both in *SF* and in *MF.* This result indicates a greater presence of slowly-decomposing substances in these leaves.

Interestingly, *C. ehrenbergiana* (Family *Celtidaceae*) had significantly higher decomposition constant ($k_2$) values of fraction *R* (both in *SF* and *MF*), suggesting a higher decomposition rate than that of the other species of the family *Fabaceae*. This result cannot be attributed to the leaf C/N ratio, since their values were statistically similar; Table 2). The fact that the non-leguminous species (*C. ehrenbergiana*) did not show significant differences from the other species indicates that it is a nutrient-demanding species (*i.e.*, it needs a high amount of available nutrients in soils). This characteristic of *C. ehrenbergiana* explains its presence in the succession only after the secondary forest has established (taking advantage of the N abundance through nitrogen fixation by the previously established leguminous species, especially *A. caven*).

Therefore, it may be attributed to the nature of organic substances (lower tannin and lignin content; Arellano *et al*., 2004; Goma-Tchimbakala & Bernhard-Reversat, 2006), which has not been evaluated in this work and should be considered in future works.

## Temporal patterns of leaf weight loss

In general, the initial phases of decomposition were rapid in the forests of the Mesopotamian Espinal, as evidenced by the high $k_1$ values, possibly due to the abundance of precipitation and relatively high summer temperature (Fig. 2) favoring lixiviation and microbial activity. This phenomenon was confirmed by Castellanos & León (2011) in a study of leaf litter decomposition in *Acacia mangium* plantations. By contrast, weight loss in the Argentine Arid *Chaco* is characterized by an initial slow phase followed by a rapid phase (Torres *et al*., 2005), with an opposite pattern in the rate of each decomposition phase, which was related to the local climatic conditions and the chemical composition of the materials.

The comparison of different species between *SF* and *MF* systems (Figs. 4b and 5) showed significant differences in the residues remaining at the end of the assay, following a decreasing order: *A. caven > P. nigra > P. affinis > C. ehrenbergiana.*

It has been reported that a faster decomposition rate involves a faster nutrient movement (Gallardo & González, 2004). Accordingly, of all the studied species, *C. ehrenbergiana* had the most active biogeochemical cycle associated with demanding species at intermediate to stable successional stages. This phenomenon might be indicating a match between decomposition rates and nutritional demands associated with productivity and litterfall contribution according to the species; if decomposition had been slow, available nutrients might have been insufficient, which would have limited plant growth and development, as reported by Montagnini & Jordán (2002) and, therefore, litterfall input.

The greatest litterfall inputs of *C. ehrenbergiana* were associated with a higher productivity and decomposition rate of the produced litterfall (Mendoza *et al.,* 2012), as opposed to the lower litterfall input of *A. caven* which, in turn, had a lower decomposition rate. Prause *et al.* (2002) observed that in Argentine *Chaco* forests, *Schinopsis balansae* Engl. was the species that most largely contributed litterfall to the soil (although it had a lower decomposition constant), compared with litterfall inputs of *P. nigra*; therefore, this system seems to be have differently from the Mesopotamian Espinal.

## Conclusions

We found significant differences, in leaf decomposition rates, between tree species that compose the forest (both in *SF* and *MF*); the remaining organic residues had, in general, the following decreasing order: *A. caven> P. nigra> P. affinis> C. ehrenbergiana.*

Considering the factors influencing litterfall decomposition processes, we conclude that the different decomposition rates observed among species are not attributable to the initial quality (C and N) of litter. The successional stage of the forests is not a factor determining decomposition rate of the most frequent species at different stages in the Mesopotamian Espinal. *C. ehrenbergiana* litter promotes a rapid decomposition in this native forest, confirming its importance for biodiversity conservation of both woody plants and native grasses, and pasture sustainability.

## References

Aceñolaza PG, 2000. Variabilidad estructural de una comunidad forestal sobre suelos vérticos de la provincia de Entre Ríos. Revista Facultad de Agronomía 20 (1): 123-130.

Aceñolaza FG, 2007. Geología y recursos geológicos de la Mesopotamia Argentina. Serie de Correlación Geológica 22: 149-155.

Aceñolaza PG, Gallardo JF, 1994. Pérdida de peso seco en hojarasca de *Alnus acuminata* en la provincia de Tucumán (Argentina). Bosque 15: 51-54. https://doi.org/10.4206/bosque.1994.v15n1-06

Arellano R, Paolini J, Vásquez L, Mora E, 2004. Producción y descomposición de hojarasca en tres agroecosistemas de café en el Estado de Trujillo, Venezuela. Revista Forestal Venezolana 48: 7-14.

Arturi M, 2006. Situación ambiental en la ecorregión Espinal. In: La situación ambiental argentina 2005; Brown A, *et al*. (eds.), pp: 241-246. Fundación Vida Silvestre Argentina, Buenos Aires.

Bueis T, Felipe Bravo F, Valentín V, Turrión MB, 2017. Influencia de la densidad del arbolado sobre el desfronde y su reciclado en pinares de repoblación del norte de España. Bosque 38(2): 401-407. https://doi.org/10.4067/S0717-92002017000200017

Bunnel FL, Tait DEN, 1974. Mathematical simulation models of decomposition: Soil organisms and decomposition in Tundra. Tundra Biome Steering Committee. pp.: 207-226. Stockholm.

Cabrera AL, 1976. Regiones fitogeográficas argentinas. Enciclopedia Argentina de Agricultura y Jardinería (Buenos Aires) 2: 1-85.

Carranza C, Noé L, Merlo C, Ledesma M, Abril A, 2012. Effect of forest clearing type on the decomposition of native and introduced pastures in the Arid Chaco, Argentina. Revista de Investigaciones Agropecuarias 38: 97-107.

Carrera AL, Mazzarino MJ, Bertiller MB, Del Valle HF, Carretero EM, 2009.Plant impacts on nitrogen and carbon cycling in the Monte phytogeographical province,

Argentina. J Arid Environ 73: 192-201. https://doi.org/10.1016/j.jaridenv.2008.09.016

Castellanos J, León JD, 2011. Descomposición de hojarasca y liberación de nutrientes en plantaciones de *Acacia mangium* (*Mimosaceae*) establecidas en suelos degradados de Colombia. Revista de Biología Tropical 59: 113-128.

Fioretto A, Di Mardo C, Papa S, Fuggi A, 2005. Lignin and cellulose degradation and nitrogen dynamics during decomposition of three leaf litter species in a Mediterranean ecosystem. Soil Biol Biochem 37: 1083-1091. https://doi.org/10.1016/j.soilbio.2004.11.007

Gallardo JF, González MI, 2004. Sequestration of C in a Spanish chestnut coppice. Invest Agrar: Sist Recur For Vol. extra: 108-113.

Gallardo JF, Merino A, 2007. El ciclo del carbono y la dinámica de los sistemas forestales. In: El papel de los bosques españoles en la mitigación del cambio climático; Bravo F (Coord.), pp: 43-64. Fundación Gas Natural, Barcelona.

Goma-Tchimbakala J, Bernhard-ReversatF, 2006. Comparison of litter dynamics in three plantations of an indigenous timber-tree species (*Terminalia superba*) and a natural tropical forest in Mayombe, Congo. Ecol Manage 229: 304-313. https://doi.org/10.1016/j.foreco.2006.04.009

INTA, 2000. Carta de Suelos de la República Argentina Departamento Villaguay, 250pp. Convenio INTA-Gobierno de Entre Ríos, Paraná (Argentina).

Jenny H, Gessel SP, Binham FT, 1949. Comparative study of decomposition rates of organic nutrients in temperate and tropical regions. Soil Sci 68: 419-432. https://doi.org/10.1097/00010694-194912000-00001

León JD, González MI, Gallardo JF, 2009. Retranslocación y eficiencia en el uso de nutrientes en bosques del centro de Antioquia. Rev CFor 12: 119-140.

León JD, González MI, Gallardo JF, 2011. Comparación del ciclo biogeoquímico en bosques naturales y plantaciones de coníferas en ecosistemas de alta montaña de Colombia. Revista de Biología Tropical 59(4): 1883-1894.

Lewis JP, Prado DE, Barberis IM, 2006. Los remanentes de bosques del Espinal en la provincia de Córdoba. In: Situación ambiental argentina 2005; Brown AD & Corcuera J (eds.), pp: 254-260. Editorial Fundación Vida Silvestre Argentina, Buenos Aires.

McCulloch CE, Searle SR, 2001. Genearalized, linear and mixed models, 4th edn. Wiley-Intersci, NY.

Mendoza C, Gallardo JF, Aceñolaza PG, Turrión MB, Pando V, 2012. Producción de hojarasca de bosques pertenecientes a una secuencia sucesional del Espinal Mesopotámico (R. Argentina). In: Aguas, suelos y vegetación en cuencas iberoamericanas; Gallardo JF (Coord.), pp.: 177-196. SIFyQA, Salamanca (Spain).

Mendoza CA, Gallardo JF, Aceñolaza PG, Turrión MB, Pando V, 2014. Temporal evolution of litterfall and potential bio-element return in a successional forest sequence of the Espinal Ecoregion, Argentina. Forest Syst 23(3): 411-424. https://doi.org/10.5424/fs/2014233-05007

Montagnini F, Jordán CF,2002. Reciclaje de nutrientes. In: Ecología y conservación de bosques neotropicales; Guariguata MR & Kattan G (eds.), pp: 167-191. Editorial Tecnológica, Cartago, Costa Rica.

Olson JS, 1963. Energy storage and balance of producers and decomposer in ecological systems. Ecology 44: 322-331. https://doi.org/10.2307/1932179

Pérez-Harguindeguy N, Díaz S, Vendramini F, Gurvich DE, Cingolani AM, Giorgis MA, Cabido M, 2007. Direct and indirect effects of climate on decomposition in native ecosystems from central Argentina. Aust Ecol 32: 749-757. https://doi.org/10.1111/j.1442-9993.2007.01759.x

Prause J, Lifschitz AP, Dalurzo HC, Agudo DE, 2002.Leaf litterfall and decomposition in a forest of the Argentine Chaco. Comm Soil Sci Plant Anal 33: 3653-3661. https://doi.org/10.1081/CSS-120015913

Prause J, Fernández López C, Contreras Leiva SM, Gallardo Lancho JF, 2012. Aporte y descomposición de hojas y reabsorción de N, P y K en un bosque primario de *Schinopsis balansae* Engl., con y sin manejo silvopastoril, en el Parque Chaqueño húmedo. Facena 28: 41-50.

Rojas AE, Saluso JH, 1987. Informe climático de la provincia de Entre Ríos. Publ. Técnica INTA EEA Paraná 14: 1-20.

Rovira P, Rovira R, 2010. Fitting litter decomposition datasets to mathematical curves: Towards a generalized. Geoderma 155: 329-343. https://doi.org/10.1016/j.geoderma.2009.11.033

SAS Inst.,2005. SAS User's Guide: Basics, version 9.1. Cary, NC, USA.

Torres PA, Abril AB, Bucher EH, 2005. Microbial succession in litter decomposition in the Semi-arid Chaco woodland. Soil Biol Biochem 37: 49-54. https://doi.org/10.1016/j.soilbio.2004.04.042

Vega Ávila AD, Toro ME, Baigori M, Fernández F, 2010. Influencia de la vegetación en la variación espacial de la abundancia de microorganismos en el desierto del Monte, San Juan, Argentina. Ecología Austral 20: 247-256.

Xu G, Hu Y, Wang S, Zhang Z, Chang X, Duan J, Luo C, Chao Z, Su A,Lin Q, Li Y, Du M, 2010. Effects of litter quality and climate change along an elevation gradient on litter mass loss in an alpine meadow ecosystem on the Tibetan Plateau. Plant Ecol 209: 257-268. https://doi.org/10.1007/s11258-009-9714-0