Temporal evolution of litterfall and potential bio-element return in a successional forest sequence of the Espinal Ecorregion, Argentina

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Abstract

Aim of study: The aim of this work was to assess the litterfall contribution and the return of bioelements of a successional forest sequence from the Mesopotamian Espinal (Argentina) which was associated with livestock production.

Area of study: Mesopotamian Espinal, Argentina.

Material and methods: Litterfall samples were taken and a chemical characterization of their fractions was determined in three stages: a) in the initial successional stage (*IF*); b) in an intermediate secondary forest (*SF*); and c) in a mature forest (*MF*).

Main results: The litterfall contribution of the three forests was $1,140 \pm 98$, $2,947 \pm 154$, and $2,911 \pm 57$ kg DM ha^{-1} yr^{-1}; respectively. The *IF* showed a seasonal pattern of contribution with a peak occurring during summer (528 ± 85 kg DM ha^{-1} yr^{-1}), then decreasing during autumn, winter, and spring (241 ± 30, 165 ± 27, and 207 ± 29 kg DM ha^{-1} season^{-1}, respectively). The *SF* showed a rather constant seasonal pattern (about 750 kg DM season^{-1}). The *MF* showed significant differences among seasons, the maximum and minimum contributions ranging between 846 ± 29 and 598 ± 33 kg DM ha^{-1} season^{-1} in summer and spring, respectively. The litterfall leaves/branch ratio decreased as ecological succession advanced, being lower as the forest gets more mature. As a consequence, this ratio can be used as an indicator of maturity in the sequence. The potential return of bio-elements of the successional forest sequence was proportional to the litterfall input, with a maximum amount of N in the *Fabaceae* species.

Research highlights: The litterfall assessment and the leaves/branch ratio allowed the characterization of the successional stages in Xerophytic forest used for livestock production.

Key words: semi-xerophytic trees; tree production pattern; plant organ contribution; leaf/branch ratio; return of bio-elements; tree nutrients.

Introduction

Litterfall production is a measure of forest productivity (Bray & Gorham, 1964; Ramírez *et al*., 2007; Aceñolaza *et al*., 2010). In particular, the analysis of litterfall has received great attention as an appropriate tool to estimate overall forest productivity (Santa Regina *et al*., 1991; Haase, 1999; Carnevale and Lewis,

2001; Zamboni & Aceñolaza, 2004; Aceñolaza *et al*., 2006).

A large portion of the organic residues that reach the soil is mineralized, including leaves, stems, flowers, fruits, and other plant structures that make up litterfall, releasing bioelements that can be reabsorbed by plants. Such nutrient release is ultimately determined by many variables as: genetic characteristics of species, annual variation of chemical composition of litterfall, microorganisms composition, and activity and environmental conditions; all these factors influen-

ce the release rate of nutrients in each forest ecosystem (Aceñolaza *et al*., 2009), determining its nutritional status.

Nutrient inputs to the soil through litterfall, is known as the potential return of bioelements (PRB), since they enter the soil after litter mineralization (Martín *et al*., 1996; Gallardo *et al*., 1989). These authors also found that, in general, temporal variations of bio-element return to the soil (via litterfall) follow an evolution similar to the pattern of litterfall contribution. Analyzing the different fractions of litterfall allows us to find some indicators of forest maturity. Martín *et al.* (1993) found that, as forest mature, the relationship between leaf and branch contribution tends to drop, even the leaf litter contribution increase, the branch production grows with a higher ratio.

The Mesopotamian Espinal has been recently modified in several areas of Entre Ríos province, due to the advance of the agricultural frontier, producing an important reduction in forest area (Aceñolaza, 2000; Muñoz *et al.,* 2005; Arturi, 2006; Maldonado *et al*., 2012) and leading to an increasingly heterogeneous land mosaic (Maldonado *et al*., 2012). Inappropriate livestock management or forest exploitation for timber production (mainly *Prosopis nigra*) or firewood (*Acacia caven* and *Prosopis affinis*) had early transformed the native and primary forests into degraded or secondary forests. Deforestation and land abandonment have led to the development of a sequence of successional forest stages, with an initial stage dominated by a woodland of *A. caven*, followed by its colonization by *P. affinis*, and leading to a mature forest dominated by *P*. *nigra*, and thus, to the reestablishment of a stable forest (Aceñolaza, 2000; Lewis *et al*., 2006).

In this study, we firstly hypothesize that the plant succession, as an ecological process, has a direct influence on the litterfall production and, as a result, on the potential nutrient return (PRB) in the forests of the Mesopotamian Espinal; secondly, that leaf/branch ratio (L/B), as a characteristic of the litter production, increases as the ecological succession advances.

This work shows data about the contribution of litterfall and quantification of PRB in Mesopotamiam Espinal forests, little-known issue until now. This subject has also a practical interest, particularly because the forests studied are associated with extensive livestock production. Therefore, the aims of this work were to:

A)   Estimate the inputs of litterfall in a degraded/restored successional sequence of the Argentine Mesopotamian Espinal eco-region.

B)   Describe the seasonal pattern of litterfall production in these forests.

C)   Quantify the potential return of bio-elements per species and forest in the successional sequence considered.

Material and methods

The Espinal is an Argentine eco-region located between 28° and 40° S latitude, to the South of the Chaqueño Park, covering approximately 330,000 km² (Lewis *et al*., 2006) and forms an arch that surrounds the Pampas Grassland eco-region. The Mesopotamian Espinal, in Entre Ríos province, comprises forests belonging to the Espinal phytogeographic Province, especially in Ñandubay district (Cabrera, 1994).

Study area

The study was conducted in the Villaguay Department (covering an area of 1,600 km² in Entre Ríos province, Argentina; Fig. 1).

The landscape was shaped by morphogenetic processes, and is currently a pen-plain, ranging from slightly undulating to plain relief, with evidences of characteristic *gilgai* micro-relief (De Petre & Stephan, 1998). Mean height is 40 m a.s.l. (INTA, 2000).

The climate in Villaguay is humid temperate. Annual mean precipitation is 1,000 mm yr^{-1}, with an important inter-annual variability (INTA, 2000), but with peaks of rainfall in autumn and spring. Mean annual temperature is about 16°C, with the mean temperature of the coldest (July) and hottest (February) months being 11 and 25°C, respectively. Temperature changes gradually from season to season; however, there may be days with minimum temperatures below 10°C in summer and with maximum values of 30°C in winter. The ombro-thermic diagram for the Villaguay Department, constructed with mean values of monthly precipitation and temperature (from 1980 to 2010), shows a drought period in February (Mendoza *et al*., 2012).

Soils are deep and characterized by a high content of expanding clays (montmorillonite), with poor drainage (Aceñolaza, 2007); they generally present calcareous concretions and low content of available phosphorous (Pav). Taxonomically, these soils correspond
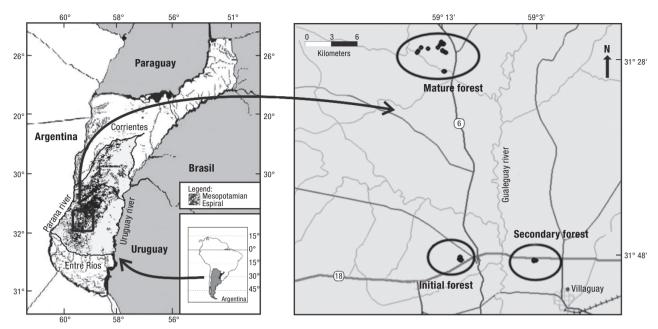
Figure 1. Location of the study area (31° 47' 59" S and 59° 11' 38" W; Entre Ríos, Argentina); the forest sites studied are delimited within the areas. The Initial forest is dominated by *Acacia caven;* Secondary forest by *Prosopis affinis;* and Mature forest by *P. nigra.*

to the order *Vertisols*, suborder *Udert*, great-group *Peludert*, sub-group *argic* (INTA, 2000).

Vegetation is typical of the Espinal phytogeographic province, Ñandubay district (Cabrera, 1994), and is characterized by dominant xerophilous tree species such as *P. nigra* (Griseb.) Hieron, *P. affinis* Spreng, *A. caven* (Molina) Molina, *Celtis ehrenbergiana* (Klotzsch) Liebm., *Aspidosperma quebracho-blanco* Schltdl., *Geoffroea decorticans* Burkart, *Schinus longifolius* (Lindl.) Speg., *Scutia buxifolia* Reissek, *Trithrinax campestris* (Burmeist.) Drude and Griseb., among other characteristic species (Burkart *et al*., 1999; INTA 1990, 2000; Aceñolaza, 2000). *P. nigra*, *P. affinis*, *A. caven*, and *G. decorticans* belong to the family *Fabaceae* and have the symbiotic capacity for N fixation.

The present study was carried out in three different forests located in similar environmental (soils and topography) and land use conditions. Considerations were taken to circumscribe the successional pattern as the main source of variation, choosing stands with similar topography, soil, and land use (kind and intensity). All stands, at the present time, have extensive livestock grazing (in rotation), with 0.4 and 0.5 units ha^{-1}, and have no logging activity. They are situated in a 20 km range and represent different successional stages (Fig. 1; INTA, 2000).

In each forest stand, four permanent plots of 800 m^2 were established to evaluate its structure and composition. All stands, in each forest category, were placed between 100 and 200 m of each other, looking for similar ecological/environmental conditions (*e. g.,* soil, topography, and land use) and avoiding closeness.

The forests selected had the following characteristics:

a) *Initial forest:* Monospecific forest of *A. caven* (*IF*), originated by colonization of this species in a cropland abandoned in 1998 (approximately 10 years at the beginnings of this study).

b) *Secondary forest:* Mixed forest (*SF*) dominated by *P. affinis*, with the presence of other characteristic species, such as *A. caven* and *C. ehrenbergiana*, which includes trees of between 50 and 70 years old.

c) *Mature forest:* Mixed old-growth forest (*MF*), dominated by *P. nigra*, with the presence of *A. caven*, *P. affinis*, and *C. ehrenbergiana*. This forest, even it presents signals of degradation (overgrazing, selected logging and fire), does not come from a clearcut area.

Climatic data

In the period when the samples were taken, temperature and rainfall were measured with a weather sta-

tion located in the study area. Mean monthly rainfall and temperature were calculated for the 2009-2010 period. Pearson's correlation coefficient (r) was used as measure of magnitude of the linear association among variables (climatic factors and total litterfall production per species and forest).

Litterfall sampling

In the three forests selected, four randomly stands (20×20 m^2) were evaluated as described before by Mendoza *et al.* (2012). Within each stand, all individual trees were identified to the species level.

Tree diameter at breast height (DBH > 5 cm) was measured, using a tape. Tree heights were calculated using a clinometer device. Tree canopy cover was estimated using the mean between N-S and E-W diameters of crowns. The main forest characteristics of the species present in *MF*, *SF*, and *IF* are showed in Table 1.

Litterfall samples were taken from the selected forest stands during 2009-2010, following the method proposed by Gauch (1982) and Aceñolaza *et al.* (2006). A total of 32 production boxes, with a 0.25 m^2 surface, were placed in December 2008. The criteria used to place boxes within the stands were under same species and only dominant woody species of each stand. In this sense, 4 boxes were placed under *A. caven* in IF, 12 boxes under SF (4 under *C. ehrenbergiana,* 4 under *A. caven*, and 4 under *P affinis*), and 16 under *MF* (4 in each of the 4 named species).

Individual boxes were randomly located under trees in the selected areas of each stand. Production boxes were placed on the soil, but separated 3 cm from soil and the bottom covered by a catching net. They were monthly sampled, beginning in January 2009 and continuing for two years.

Litterfall samples were collected from each box in different paper bags that were previously labeled at the lab. Later they were dried in a 70°C heater until a constant weight was reached; then these samples were classified and weighed, separating the following fractions: a) leaves; b) branches; c) flowers; d) fruits; and d)

Table 1. Soil and structural characteristics of the three forest stand studied. Initial forest (*IF*), secondary forest (*SF*), and mature forest (*MF*). Soil characteristics derived from unpublished data of main author doctoral research

	IF	*SF*	*MF*
Latitude	31° 48' 26" S	31° 47' 46" S	31º 28' 11" N
Longitude	59° 11' 23" O	59° 4' 14" O	59°14'20"O
Dominant specie	*A. caven*	*P. affinis*	*P. nigra*
Other specie		*A. caven;*	*A. caven;*
		C. ehrenbergiana	*C. ehrenbergiana*
			P. affinis
Soil type	*Vertisols*	*Vertisols*	*Vertisols*
Sand (%)	19	18	21
Silt (%)	24	26	30
Clay (%)	50	49	42
pH	6.0	5.8	5.8
Soil organic matter (%)	6.6	7.5	9.5
Soil organic C (g/kg)	38.1	43.5	55.2
Total N (g/kg)	3.1	3.7	4.3
C/N	12.3	11.8	12.8
Avail. P (mg/kg)	8.4	11.8	18.9
Total Ca (g/kg)	6.2	6.8	6.6
Total Mg (mg/kg)	508	518	517
Total K (mg/kg)	514	499	605
Canopy cov. (%)	61 (±12)	80 (±19)	92 (±21)
DBH (cm)	16.3 (±5.4)	33.2 (±11.4)	32.4 (±10.4)
Basal area (m^2)	5.5 (±1.2)	8.4 (±5.4)	9.1 (±3.1)
Density (trees/ha)	373 (±54)	227 (±48)	116.5 (±42)
Height (m)	3.4 (±0.8)	4.3 (±1.5)	5.6 (±1.1)

others (other plant material from other tree, shrub or herb species, excluding animal feces).

Results of annual litter production are expressed in kg of dry matter (DM) ha^{-1} yr^{-1}.

Total litterfall, litterfall fraction, and moisture content of each production box were determined per species and per forest as a function of time. Total litterfall was calculated as the average of the annual accumulation of litterfall production.

Collecting trays were placed under individual species. For referring the litterfall production to ha, an adjustment of litter input values by tree should be performed, to fit the values measured to the actual coverage of each species in the area. For this, the relative tree cover on each species (Mendoza *et al.*, 2012) was multiplied by the litterfall (resulting kg MS ha^{-1} y^{-1}). This allowed us to estimate the real contribution by species and site. In the forests more than one species are found, total contributions is obviously the sum of the relative contributions of each species.

Chemical analyses of litterfall samples

All plant samples were ground using a ball mill (Retsch, model MM301). These samples were then weighed using a precision balance ($\pm$ 0.001 g) and burned in a muffle furnace (at least 500°C) for 5 h.

Ashes were digested with concentrated HCl and then diluted with distilled water to a known volume. Aliquots were taken to determine P, using the vanadomolybdate yellow procedure (Chapman & Pratt, 1979) and a spectrophotometer (Genesys, model 20). Another aliquot of the same acidic solution was used to determine Ca, Mg, K, S, Na, Fe, Al, Zn, Mn, and Cu by means of inductively coupled plasma emission spectrometry (ICP-OES).

After the acid digestion of plant samples, N content was determined using a flow auto-analyzer (Bran+ Luebbe AA3).

Quantification of potential return of bio-elements (PRB)

The amount of bioelements that potentially can return to the soil through litterfall was determined by multiplying the pooled mean annual litterfall production estimated during the study period by the mean chemical composition of each plant residue. Then, PRB was calculated for each fraction per species and per forest.

Statistical analyses

Temporal analysis included average litterfall production accumulated by month and season (summer: January-March, autumn: April-June, winter: July-September, and spring: October-December).

Data were analyzed using a linear mixed model analysis of variance (McCulloch & Searle, 2001) with two between-subject factors in a hierarchical design (forest and species nested within forest) and three within-subject factors of repeated measures (year, season, and month nested within season). The comparison between means was done by using Fisher's Least Significant Difference (LSD) test.

For each fixed forest, year, and season, covariance among months is constant for a single species and equal for all species. Therefore, the structure of variances of the mixed linear model was estimated using the restricted maximum likelihood method (Bartlett, 1937).

The model proposed allowed us to estimate the actual contribution of the different forests as a function of the entire forest tree cover and the relative cover per species in the three stands representative of the Mesopotamian Espinal (Mendoza *et al.*, 2012).

Results

Total litter production in the forest systems

Total litterfall annual production

Mean forest litterfall contribution (Table 2) ranged between 1,140 kg DM ha^{-1} yr^{-1} in *IF* and 2,947 kg DM ha^{-1} yr^{-1} in *SF*, showing no significant differences with *MF* (2,911 kg DM ha^{-1} yr^{-1} in MF, $p > 0.05$). However, both *SF* and *MF* differed statistically from *IF* ($p < 0.05$).

Total litterfall seasonal production

Comparison of forests seasonal patterns (Table2) showed significant differences among forests in summer ($p < 0.05$); *SF* and *MF* exhibited the greatest production, without significant differences between both them ($p > 0.05$). The same pattern was observed

Table 2. Total litterfall production by forest and season during 2009-2010. Results are expressed in kg DM ha⁻¹ yr⁻¹ (±standard error). The Initial forest (*IF*), Secondary forest (*SF*), and Mature forest (*MF*) are indicated. Different capital letters indicate significant differences ($p < 0.05$) between forests (total and seasonal values); different small letters indicate significant differences among seasons within a forest ($p < 0.05$). Fisher's LSD test was used for comparisons of means; n = number of sample

Forests	n	Summer	Autumn	Winter	Spring	Total forest
IF	96	528 (±85)[Ba]	241 (±30)[Bb]	165 (±27)[Cb]	207 (±29)[Cb]	1,140 (±98)[B]
SF	288	787 (±66)[Aa]	768 (±70)[Aa]	637 (±90)[Ba]	755 (±79)[Aa]	2,947 (±154)[A]
MF	384	846 (±29)[Aa]	703 (±24)[Ac]	764 (±27)[Ab]	598 (±33)[Bd]	2,911 (±57)[A]

in autumn, *SF* and *MF* having the maximum production in summer and autumn.

IF, *SF*, and *MF* showed significant differences ($p < 0.05$) in total litterfall production in the three forests in winter and spring. In winter, *IF*, *SF*, and *MF* produced 165, 637, and 764 kg DM ha⁻¹ season⁻¹, respectively; however, in spring, *SF* showed maximum production, with values of 207, 755, and 598 kg DM ha⁻¹ season⁻¹ for *IF*, *SF*, and *MF*, respectively (Table 2). The analysis of the temporal pattern exhibited a maximum contribution in summer for *IF* (528 kg DM ha⁻¹ season⁻¹), with significant differences ($p < 0.05$) from the other seasons (Table 2). *SF* shows constant production ($p > 0.05$). Finally, *MF* presented significant differences among seasons ($p < 0.05$), with the maximum and the minimum seasonal litterfall production ranging between 846 and 598 kg DM ha⁻¹ season⁻¹ (summer and spring, respectively; Table 2).

Total litterfall production by species

Comparing the total litterfall production by species, the maximum contributions were found in *A. caven*

(*IF*), *C. ehrenbergiana*, and *P. nigra* (MF), without significant differences among them ($p > 0.05$); even that they were different compared to the minimum contribution of *A. caven* in the *MF* (Table 3).

Temporal litterfall pattern was analyzed considering mean contribution per species (Table 3); these data are referred to monthly means, and did not show a definite pattern.

As already mentioned, *A. caven* showed a decreasing seasonal production pattern in *IF*, with a maximum value of 528 kg DM ha⁻¹ season⁻¹ in summer, which differed significantly from the other three seasons ($p < 0.05$).

Production of *P. affinis* was constant and did not differ significantly among seasons in *SF* ($p > 0.05$), whereas *A. caven* and *C. ehrenbergiana* showed opposite contribution patterns among seasons compared to *P. affinis*; this is the reason why, when both are considered in combination, the total production follows an annual decreasing rate (Table 3).

Following the same trend, the contribution patterns of *P. nigra* and *C. ehrenbergiana* were complementary in *MF*, *i. e.*, they were opposite among seasons; the same pattern was also observed between *C. ehrenbergiana* and *A. caven* in *SF*. Whereas *P. nigra* had the ma-

Table 3. Total litterfall production (kg DM ha⁻¹ yr⁻¹) by species and its mean seasonal values (±standard error), for *A. caven; P. affinis, C. ehrenbergiana,* and *P. nigra* in Initial forest (*IF*), Secondary forest (*SF*) and Mature forest (*MF*), during the period studied (2009 and 2010). Letters of significance ($p < 0.05$) are included. Capital letters indicate comparison among species per forest; small letters indicate comparison among seasons. Fisher's LSD test was used for comparisons of means, n = number of sample

Forest	Species	n	Summer	Autumn	Winter	Spring	Total
IF	*A. caven*	96	528 (±84.7)[Aa]	241 (±30.2)[Cb]	165 (±26.5)[Bb]	207 (±29.3)[Bb]	1,140 (±98.2)[A]
SF	*A. caven*	96	323 (±55.4)[Ba]	193 (±58.3)[Cb]	150 (±75.5)[Bb]	315 (±66.5)[Aa]	982 (±129)[B]
	P. affinis	96	278 (±66.1)[Ba]	252 (±69.8)[Ba]	268 (±89.9)[Ba]	239 (±79.4)[Aa]	1,037 (±154)[B]
	C. ehrenbergiana	96	186 (±21.4)[Cb]	323 (±22.6)[Ba]	219 (±29.2)[Bb]	201 (±25.7)[Bb]	928 (±49.8)[B]
MF	*A. caven*	96	57.5 (±8.9)[Ea]	36.8 (±6.8)[Eb]	23.1 (±8.1)[Db]	29.7 (±10.1)[Cb]	147 (±17.2)[D]
	P. affinis	96	108 (±19.9)[Da]	59.5 (±14.7)[Db]	72.0 (±17.4)[Cab]	54.2 (±21.6)[Cb]	294 (±37.1)[C]
	C. ehrenbergiana	96	289 (±29.2)[Bb]	395 (±23.8)[Aa]	409 (±27.2)[Aa]	168 (±32.7)[Bc]	1,260 (±56.8)[A]
	P. nigra	96	391 (±58.7)[Aba]	212 (±45.9)[Cb]	260 (±54.7)[Bab]	3,467 (±67.9)[Aa]	1,210 (±115)[A]

ximum production in the summer-spring period, *C. ehrenbergiana* did in autumn-winter, with their maximum and minimum values showing significant differences ($p < 0.05$; Table 3).

The comparison between productions of a single species among forests showed that *A. caven* (both in *MF* and *IF*) also had a decreasing pattern (with a maximum in summer); additionally, the maximum for *A. caven* in *SF* was in spring/summer.

P. affinis showed a constant production pattern in both *MF* and *SF*; however, the recorded contribution was lower in *MF*, probably due also to the competition of dominant species in these forests (*P. nigra* and *C. ehrenbergiana*).

C. ehrenbergiana showed a production pattern typical of deciduous species in both SF and MF, with peaks in autumn in both ecosystems.

Overall, comparison of litterfall productions shows significant differences among species and among seasons (Table 3).

Litterfall production by fractions

The comparison of litterfall production per fraction showed that leaves contributed with unusually low values in all cases, exhibiting maximum values for *C. ehrenbergiana* in *MF* (mean of 652 kg DM ha^{-1} yr^{-1}) and minimum values for *A. caven* in *MF* (mean 22 kg DM ha^{-1} yr^{-1}), with significant differences between them ($p < 0.05$; Table 4), ranging from 52 to 15% of the litterfall.

Branches showed very dissimilar contribution values, with a maximum of 710 kg DM ha^{-1} yr^{-1} produced by *P. nigra* and a minimum of 76 kg DM ha^{-1} yr^{-1} by *A. caven*

Table 4. Total mean and litterfall contribution considering the different components (leaves, branches, flowers, fruits, and "others"), per forest and species (kg DM ha^{-1} yr^{-1} ±standard error) and leaves/branches relationship (L/B). The Initial forest (*IF*), secondary forest (*SF*) and mature forest (*MF*) are indicated. The relative percentages (below, in bold and italics) are also indicated. Different letters indicate significant differences ($p < 0.05$) between lines. Fisher's LSD test was used for comparisons of means, n = number of sample

Forest	Species	n	Year	Leaves	Branches	Flowers	Fruits	Others	Total	L/B
IF	A. caven	96	mean	345 (±67.1)^C	195 (±54.9)^C	12.2 (±6.1)^B	330 (±61.0)^A	256 (±42.7)^A	1,140 (±98.2)^A	1.8
			%	*30.2*	*17.1*	*1.1*	*28.9*	*22.5*	*100*	
	A. caven	96	mean	226 (±109)^C	457 (±102)^B	14.0 (±2.8)^B	76.0 (±31.0)^B	209 (±37.2)^A	982 (±129)^B	0.5
			%	*23.1*	*46.6*	*1.4*	*7.7*	*21.3*	*100*	
SF	P. affinis	96	mean	431 (±104)^B	305 (±118)^C	3.7 (±3.7)^C	29.6 (±12.7)^B	266 (±44.4)^A	1,037 (±154)^B	1.4
			%	*41.5*	*29.4*	*0.4*	*2.9*	*25.7*	*100*	
	C. ehrenbergiana	96	mean	509 (±42.0)^B	199 (±38.4)^C	25.8 (±1.2)^A	34.2 (±12.0)^B	160 (±14.4)^B	928 (±49.8)^B	2.6
			%	*54.8*	*21.4*	*2.8*	*3.7*	*17.2*	*100*	
	A. caven	96	mean	21.7 (±15.4)^E	76.0 (±16.8)^D	0.7 (±0.7)^C	2.8 (±2.8)^C	46.9 (±9.1)^C	147 (±17.2)^D	0.3
			%	*14.8*	*51.7*	*0.5*	*1.9*	*31.9*	*100*	
MF	P. affinis	96	mean	74.3 (±33.0)^D	152 (±36.0)^C	0	0.8 (±0.8)^C	66.0 (±19.5)^C	294 (±37.1)^C	0.5
			%	*25.3*	*51.5*	*0*	*0.3*	*22.4*	*100*	
	C. ehrenbergiana	96	mean	652 (±51)^A	307 (±53)^B	1.2 (±1.2)^C	39.1 (±23.0)^B	253 (±32)^A	1,260 (±57)^A	2.1
			%	*51.7*	*24.4*	*0.1*	*3.1*	*20.1*	*100*	
	P. nigra	96	mean	308 (±103)^C	710 (±108)^A	16.5 (±12.7)^B	23.5 (±23.0)^B	153 (±61)^B	1,210 (±115)^A	0.4
			%	*25.4*	*58.6*	*1.4*	*1.9*	*12.6*	*100*	

(both in *MF*), with significant differences between them ($p < 0.05$; Table 4). These contributions represented 59 and 52% of the total production, respectively.

Flowers were residual, with the most important production belonging to *C. ehrenbergiana* in *SF* (26 kg DM ha^{-1} yr^{-1}), and differing statistically from the contribution of the other species to the total; this maximum corresponded to the highest representation of the flower fraction among species (3%).

The highest fruit production corresponded to *A. caven* in *IF*, with a mean of 330 kg DM ha^{-1} yr^{-1}. Fruits of *A. caven* represented 29, 7.7, and 1.9% in *IF*, *SF*, and *MF*, respectively (Table 4).

Finally, contribution of the fraction "other" was highest in *P. affinis* in *SF*, *C. ehrenbergiana* in *MF*, and *A. caven* in *IF* and *SF* ($p > 0.05$; Table 4), showing significant differences from the minimum production, which corresponded to *C. ehrenbergiana* in *SF*, and *A. caven* and *P. affinis* in *MF*.

This index L/B varied widely among species (Tabla 4), with high values of 2.1 and 2.6 recorded for *C. ehrenbergiana* in *MF* and *SF*, respectively; values of about 0.5 for *A. caven* in *SF* and *MF*, and *P. affinis* or *P. nigra* in *MF*; and intermediate values of 1.8 for *A. caven* in IF or 1.4 for *P. affinis* in *SF*.

Correlations between litterfall production and climatic parameters

The current analysis was conducted in forests of the Mesopotamian Espinal and no significant correlations were observed between monthly production and climatic factors, such as precipitation ($r^2 < 0.01$; $p > 0.72$) and temperature ($r^2 = 0.01$; $p > 0.12$).

Total potential return of bioelements (PRB)

Macro-elements (N, Ca, K, Mg, P and S) and micro-elements (Na, Fe, Al, Zn, Mn and Cu) were proportional to the litterfall production of the successional sequence. Total PRB values were 37.1, 140, and 150 kg ha^{-1} yr^{-1} in *IF*, *SF*, and *MF*, respectively, whereas micro-elements were 0.5, 1.8, and 1.9 kg ha^{-1} yr^{-1} in *IF*, *SF*, and *MF*, respectively (Table 5). The order of quantitative importance of total bioelements returned by each forest was the following:

IF: N > Ca > K > Mg > P > S >> Na > Fe > Al > Zn > Mn > Cu;
SF: Ca > N > K > Mg > P > S >> Na > Fe > Al > Mn > Zn > Cu;
and
MF: Ca > N > K > Mg > P > S >> Na > Fe > Al > Mn > Zn > Cu.

Potential return of bioelements (PRB) per species in each forest

Maximum total PRB was observed for *C. ehrenbergiana* in *MF*, with a total of 82.6 kg ha^{-1} yr^{-1} of bioelements, whereas the minimum value was recorded for *A. caven* also in *MF*, with only 4.2 kg ha^{-1} yr^{-1}. In the remaining species, PRB ranged between 11.1 and 68.6 kg ha^{-1} yr^{-1} (Table 5).

PRB for *A. caven* (Table 5) differed among *IF*, *SF*, and *MF* (37.6, 33.4, and 4.2 kg ha^{-1} yr^{-1} of total bio-

Table 5. Potential return of bio-elements (PRB) per species and forest (2009-2010). Mean dry matter (DM), N, P, K, Ca, Mg, and S (values expressed in kg ha^{-1} yr^{-1} ±standard error) returns; and Al, Cu, Fe, Mn, Na, and Zn (values expressed in g ha^{-1} yr^{-1} ±standard error) returns. Totals (in bold) refer to initial forest (*IF*), secondary forest (*SF*) and mature forest (*MF*) per species and nutrients

Species	DM	N	P	K	Ca	Mg	S	Al	Cu	Fe	Mn	Na	Za	Total
A. caven	882 (±356)	18.5 (±5.6)	0.8 (±0,5)	3.5 (±1,2)	13.0 (±4,7)	0.9 (±0,4)	0.5 (±0,2)	104 (±43)	12 (±5)	120 (±51)	24 (±8)	180 (±75)	24 (±5)	38 (±15)
Total *IF*	**882 (±356)**	**18.5 (±5.6)**	**0.8 (±0,5)**	**3.5 (±1,2)**	**13.0 (±4,7)**	**0.9 (±0,4)**	**0.5 (±0,2)**	**104 (±43)**	**12 (±5)**	**120 (±51)**	**24 (±8)**	**180 (±75)**	**24 (±5)**	**38 (±15)**
A. caven	774 (±321)	15.1 (±4,5)	0.5 (±0,2)	1.7 (±0,6)	14.4 (±4,6)	0.7 (±0,3)	0.4 (±0,2)	97 (±38)	11 (±6)	109 (±46)	21 (±8)	168 (±61)	23 (±8)	33 (±12)
P. affinis	769 (±286)	18.5 (±3,7)	0.6 (±0,3)	2.1 (±56)	16.3 (±5,1)	0.8 (±0,4)	0.6 (±0,3)	132 (±61)	18 (±8)	153 (±68)	54 (±17)	220 (±83)	45 (±16)	40 (±17)
C. ehrenbergiana	770 (±214)	19.0 (±5,1)	0.9 (±0,4)	3.1 (±56)	42.8 (±13,2)	1.6 (±0,5)	0.5 (±0,3)	126 (±41)	20 (±9)	182 (±71)	83 (±31)	249 (±79)	43 (±21)	69 (±27)
Total *SF*	**2,313 (±536)**	**52.7 (±13,2)**	**2.1 (±0,9)**	**6.9 (±56)**	**73.4 (±22,4)**	**3.1 (±1,2)**	**1.6 (±0,8)**	**400 (±135)**	**50 (±23)**	**400 (±167)**	**200 (±63)**	**600 (±225)**	**100 (±42)**	**142 (±58)**
A. caven	101 (±47)	1.8 (±0,6)	0.1 (±0,1)	0.2 (±0,1)	2.0 (±0,6)	0.1 (±0,1)	0	14 (±5)	2 (±1)	15 (±6)	3 (±1)	24 (±11)	3 (±1)	4 (±3)
P. affinis	227 (±71)	4.8 (±1,2)	0.1 (±0,1)	0.6 (±0,2)	5.0 (±1,7)	0.2 (±0,1)	0.2 (±0,1)	32 (±12)	4 (±2)	36 (±12)	13 (±6)	54 (±23)	11 (±4)	11 (±5)
C. ehrenbergiana	999 (±342)	25.0 (±5,8)	0.1 (±0,1)	3.8 (±1,3)	48.8 (±15,1)	2.2 (±1,7)	0.8 (±0,3)	177 (±48)	28 (±12)	253 (±104)	115 (±49)	348 (±125)	62 (±23)	83 (±28)
P. nigra	1,058 (±458)	24.0 (±4,7)	0.9 (±0,3)	3.5 (±1,4)	23.2 (±9,8)	0.9 (±0,3)	0.9 (±0,4)	112 (±37)	21 (±9)	178 (±76)	75 (±32)	252 (±116)	50 (±17)	54 (±21)
Total *MF*	**2,385 (±856)**	**55.6 (±13,4)**	**2.1 (±0,6)**	**8.0 (±3,1)**	**79.0 (±25,9)**	**3.3 (±2,2)**	**2.0 (±0,8)**	**300 (±102)**	**100 (±45)**	**500 (±215)**	**200 (±82)**	**700 (±347)**	**100 (±48)**	**152 (±58)**

elements, respectively), but showed the same descending order of contribution of the following elements to the total:

N > Ca > K > Mg > P > S >> Na > Fe > Al > Zn > Mn > Cu

P. affinis (Table 5) returned 39.6 kg ha⁻¹ yr⁻¹ in *SF* and 11.1 kg ha⁻¹ yr⁻¹ in *MF* of total bioelements; however, there were differences in the amounts of nutrient return and in the order of importance of the total return. The descending order of element contribution in *SF* was:

N > Ca > K > Mg > P > S >> Na > Fe > Al > Mn > Zn > Cu

Whereas the descending order in *MF* was:

Ca > N > K > Mg > S > P >> Na > Fe > Al > Mn > Zn > Cu

The establishment of the primary forests generated displacement of N by Ca concerning the contribution of these two bioelements to the total PRB.

C. ehrenbergiana (Table 5) had maximum contribution in *SF* and *MF* (82.6 and 68.6 kg ha⁻¹ yr⁻¹, respectively) and the elements followed the same descending order of contribution in both forests:

Ca > N > K > Mg > P > S >> Na > Fe > Al > Mn > Zn > Cu

Finally, *P. nigra* (Table 5) produced 54 kg ha⁻¹ yr⁻¹ in *MF*, with the bioelement contribution following descending order:

N > Ca > K > S > P > Mg >> Na > Fe > Al > Mn > Zn > Cu

PRB considering litterfall fractions and distribution among forests

The maximum contribution of the leaf fraction to the PRB was found in *SF*, with 86.4 kg ha⁻¹ yr⁻¹, whereas maximum PRB contribution of branches was found in *MF*, with only 60.6 kg ha⁻¹ yr⁻¹ (Table 6). PRB contribution of flowers to the PRB was negligible, ranging from 0.8 to 3.5 kg ha⁻¹ yr⁻¹ in *IF* and *SF*, respectively, *MF* showing intermediate values. Fruits had a maximum contribution to PRB in *IF* (with 12.3 kg ha⁻¹ yr⁻¹), followed by *SF* and *MF* (with 9.0 and 6.7 kg ha⁻¹ yr⁻¹, respectively).

The analysis of PRB values among species showed that the minimum and maximum N return by leaves was found in the leaf fraction in *MF* (0.6 and 17.1 kg N ha⁻¹ yr⁻¹ in *A. caven* and *C. ehrenbergiana*, respectively); obviously, the dominant factor seems to be production.

Branches had a minimum return of 1.2 kg N ha⁻¹ yr⁻¹ (*A. caven* in *MF*), reaching a maximum of 14.0 kg ha⁻¹

yr⁻¹ (*P. nigra* also in *MF*), according to the dominance of the latter species.

Maximum N in flowers was found for *P. nigra*, the remaining species having a negligible contribution.

Finally, fruits had a maximum contribution of 6.7 kg N ha⁻¹ yr⁻¹ for *A. caven* in *IF*.

In general, P return exhibited very low values (ranging between 0.7 and 0.1 kg P ha⁻¹ yr⁻¹; Table 6). Maximum contribution of K, Ca, and Mg was provided by the leaf fraction of *C. ehrenbergiana* (both in *SF* and *MF*). K return of the fruit fraction was more than double the contribution of the leaf fraction for *A. caven* in *IF*; branches contributed proportionally more Ca, whereas Al and Na were important in the flower fraction.

P. affinis in *SF* showed an important Al return through the branch and flower fractions, whereas Cu, Na, and Zn were only important in flowers, and Mn in branches; the remaining elements were proportional to the DM amount returned. In addition, in *MF* this species made significant contributions of Al, Cu, Fe, Na, and Zn through flowers (Table 6).

Discussion

Annual forest litterfall contribution by species

The litterfall production in forests of the Mesopotamian Espinal studied are below the mean ones recorded for warm-temperate forests, although higher than those found in some meso-thermal subtropical forests. Annual forest litterfall was somewhat lower than the mean global estimation (5,600 kg DM ha⁻¹ yr⁻¹; Brinson *et al*., 1980) for warm-temperate forests. However, the three forests studied exceed the value of 300 kg DM ha⁻¹ yr⁻¹ indicated by Carnevale and Lewis (2001) for other meso-thermal subtropical forests studied in northern Argentina.

In this study, we explored the hypothesis that litterfall contribution increases as ecological succession progresses. A significantly greater annual production, both in *SF* and *MF*, than in *IF* was recorded. Maximum production was expected in *MF*, but the value found did not differ significantly from *SF*, probably because the canopy cover has become constant, with little variation between *SF* and *MF*. This fact partly confirms our hypothesis, since productivity of ecosystems of the Mesopotamian Espinal became stable at the *SF* stage.

Table 6. Potential return of bio-elements (PRB) considering the main contributing fractions (leaves, branches, flowers, and fruits). Mean dry matter (DM), N, P, K, Ca, Mg, and S (values expressed in kg ha^{-1} yr^{-1} ±standard error) returns; and Al, Cu, Fe, Mn, Na and Zn (values expressed in g ha^{-1} yr^{-1} ±standard error) returns. Totals (in bold) refer to initial forest (*IF*), secondary forest (*SF*), and mature forest (*MF*), per fractions, species and nutrients

Fractions	Forest	Species	MS	N	P	K	Ca	Mg	S	Al	Cu	Fe	Mn	Na	Zn	Total
Leaves	IF	*A. caven*	345 (±56)	8.5 (±0.8)	0.4 (±0.1)	0.9 (±0.1)	6.2 (±0.6)	0.4 (±0.1)	0.2 (±0.1)	30 (±11)	1.9 (±0.2)	45 (±13)	8 (±2)	36 (±11)	6 (±1)	17 (±10)
	SF	*A. caven*	226 (±125)	6.2 (±0.8)	0.3 (±0.1)	0.7 (±0.1)	3.7 (±2.2)	0.3 (±0.1)	0.2 (±0.1)	20 (±9)	2.1 (±0.4)	29 (±11)	5 (±2)	24 (±9)	4 (±1)	12 (±5)
		P. affinis	431 (±232)	12.6 (±0.8)	0.4 (±0.1)	1.3 (±0.1)	9.2 (±2.2)	0.5 (±0.1)	0.5 (±0.1)	35 (±12)	5.2 (±0.4)	51 (±11)	13 (±5)	54 (±19)	13 (±2)	25 (±12)
		C. ehrenbergiana	509 (±214)	12.6 (±0.8)	0.7 (±0.1)	2.4 (±0.2)	32.8 (±2.2)	1.3 (±0.1)	0.4 (±0.1)	40 (±15)	3.8 (±0.4)	67 (±11)	32 (±9)	80 (±27)	8 (±2)	50 (±21)
		Total *SF*	**1,167 (±571)**	**31.3 (±2.5)**	**1.4 (±0.2)**	**4.4 (±0.4)**	**45.7 (±6.5)**	**2.1 (±0.3)**	**1.1 (±0.3)**	**95 (±26)**	**11.1 (±1.1)**	**147 (±32)**	**50 (±16)**	**158 (±39)**	**25 (±4)**	**86 (±32)**
	MF	*A. caven*	22 (±12)	0.6 (±0.6)	0	0.1 (±0.0)	0.3 (±1.8)	0	0	2 (±1)	0.1 (±0.1)	3 (±1)	0	2 (±1)	0	1 (±1.0)
		P. affinis	74 (±54)	2.1 (±0.7)	0.1 (±0.0)	0.3 (±0.1)	1.6 (±1.8)	0.1 (±0.1)	0.1 (±0.1)	6 (±2)	0.9 (±0.1)	29 (±6)	2 (±1)	9 (±2)	2 (±1)	4 (±2.0)
		C. ehrenbergiana	652 (±312)	17.1 (±0.7)	0.7 (±0.1)	3.1 (±0.1)	36.1 (±1.8)	1.9 (±0.1)	0.6 (±0.1)	52 (±8)	5.1 (±0.2)	86 (±16)	41 (±8)	102 (±18)	10 (±2)	60 (±24)
		P. nigra	308 (±125)	8.7 (±0.6)	0.4 (±0.1)	1.4 (±0.1)	6.2 (±1.7)	0.4 (±0.1)	0.4 (±0.1)	23 (±7)	2.9 (±0.1)	33 (±9)	8 (±2)	50 (±17)	8 (±1)	18 (±7)
		Total *MF*	**1,056 (±487)**	**28.5 (±2.6)**	**1.2 (±0.2)**	**4.9 (±0.3)**	**44.2 (±7.1)**	**2.3 (±0.3)**	**1.1 (±0.3)**	**82 (±21)**	**9.0 (±0.4)**	**131 (±26)**	**52 (±11)**	**163 (±39)**	**20 (±4)**	**83 (±32)**
Branches	IF	*A. caven*	195 (±86)	2.6 (±0.5)	0.1 (±0.0)	0.2 (±0.3)	4.2 (±0.8)	0.2 (±0.1)	0.1 (±0.1)	23 (±9)	4.2 (±0.3)	33 (±10)	8 (±1)	39 (±11)	6 (±2)	8 (±3.0)
	SF	*A. caven*	457 (±189)	6.7 (±1.2)	0.2 (±0.1)	0.5 (±0.1)	10.0 (±1.8)	0.4 (±0.1)	0.2 (±0.1)	24 (±11)	3.8 (±0.6)	34 (±14)	8 (±2)	40 (±18)	6 (±3)	18 (±7.0)
		P. affinis	305 (±178)	5.4 (±1.2)	0.2 (±0.1)	0.5 (±0.1)	6.9 (±1.8)	0.2 (±0.1)	0.2 (±0.1)	47 (±20)	4.0 (±0.6)	42 (±17)	18 (±2)	58 (±12)	8 (±4)	14 (±6)
		C. ehrenbergiana	199 (±76)	4.5 (±1.2)	0.1 (±0.0)	0.3 (±0.1)	5.6 (±1.8)	0.1 (±0.1)	0.1 (±0.1)	28 (±13)	3.0 (±0.6)	46 (±19)	14 (±2)	48 (±2)	7 (±3)	11 (±4.0)
		Total *SF*	**961.1 (±278)**	**16.9 (±3.6)**	**0.5 (±0.4)**	**1.3 (±0.3)**	**22.5 (±5.4)**	**0.7 (±0.2)**	**0.5 (±0.3)**	**99 (±35)**	**11.0 (±1.8)**	**122 (±58)**	**40 (±7)**	**146 (±37)**	**21 (±10)**	**43 (±16)**
	MF	*A. caven*	76 (±34)	1.2 (±1.0)	0	0.1 (±0.1)	1.7 (±1.6)	0.1 (±0.1)	0	4 (±2)	1.1 (±0.8)	5 (±2)	1 (±1)	6 (±1)	1 (±1)	3 (±1.0)
		P. affinis	152 (±89)	2.7 (±1.1)	0.1 (±0.0)	0.3 (±0.1)	3.4 (±1.6)	0.1 (±0.1)	0.1 (±0.1)	13 (±8)	0.9 (±0.8)	11 (±7)	5 (±3)	16 (±3)	2 (±1)	7 (±3.0)
		C. ehrenbergiana	307 (±203)	6.7 (±1.1)	0.2 (±0.1)	0.4 (±0.1)	8.7 (±1.6)	0.2 (±0.1)	0.2 (±0.1)	40 (±18)	4.2 (±1.0)	66 (±14)	21 (±3)	70 (±13)	10 (±4)	17 (±8.0)
		P. nigra	710 (±302)	13.3 (±1.0)	0.5 (±0.1)	1.7 (±0.1)	16.7 (±1.6)	0.5 (±0.1)	0.4 (±0.2)	36 (±14)	3.8 (±0.8)	59 (±15)	18 (±3)	63 (±23)	9 (±4)	34 (±12)
		Total *MF*	**1,244 (±452)**	**24.9 (±4.1)**	**0.8 (±0.2)**	**2.5 (±0.5)**	**30.5 (±6.2)**	**0.9 (±0.3)**	**0.7 (±0.4)**	**93 (±38)**	**10.0 (±3.5)**	**141 (±58)**	**45 (±12)**	**155 (±42)**	**23 (±10)**	**61 (±26)**
Flowers	IF	*A. caven*	12 (±9)	0.4 (±0.1)	0	0	0.1 (±0.1)	0	0	48 (±13)	3 (±1)	33 (±12)	4 (±2)	72 (±22)	6 (±2)	0.8 (±0.3)
	SF	*A. caven*	14 (±7)	0.5 (±0.1)	0	0	0.2 (±0.1)	0	0	49 (±11)	3 (±1)	35 (±15)	4 (±2)	74 (±25)	6 (±2)	0.9 (±0.4)
		P. affinis	4 (±1)	0.1 (±0.1)	0	0	0.1 (±0.1)	0	0	41 (±15)	7 (±3)	38 (±16)	11 (±5)	83 (±35)	18 (±6)	0.4 (±0.2)
		C. ehrenbergiana	26 (±13)	1.0 (±0.1)	0.1 (±0.1)	0.3 (±0.1)	0.6 (±0.1)	0.1 (±0.1)	0	41 (±9)	7 (±4)	38 (±21)	11 (±6)	83 (±42)	18 (±7)	2.2 (±0.9)
		Total *SF*	**44 (±21)**	**1.6 (±0.4)**	**0.1 (±0.1)**	**0.3 (±0.1)**	**0.9 (±0.5)**	**0.1 (±0.1)**	**0**	**131 (±25)**	**17 (±9)**	**111 (±6)**	**26 (±12)**	**240 (±92)**	**42 (±15)**	**3.5 (±1.2)**
	MF	*A. caven*	1 (±1)	0	0	0	0	0	0	8 (±3)	0	5 (±2)	1 (±1)	11 (±9)	1 (±2)	0
		P. affinis	1 (±1)	0	0	0	0	0	0	11 (±5)	2 (±1)	10 (±4)	3 (±2)	22 (±12)	5 (±3)	0.1 (±0.1)
		C. ehrenbergiana		0	0	0	0	0	0	60 (±21)	10 (±4)	55 (±22)	16 (±7)	121 (±45)	26 (±12)	0.3 (±0.1)
		P. nigra	16 (±13)	0.7 (±0.2)	0	0.2 (±0.1)	0.1 (±0.1)	0	0	31 (±25)	6 (±3)	44 (±12)	16 (±5)	90 (±51)	19 (±9)	1.2 (±0.7)
		Total *MF*	**19 (±18)**	**0.5 (±0.2)**	**0.1 (±0.1)**	**0.2 (±0.1)**	**0.1 (±0.1)**	**0**	**0**	**110 (±57)**	**18 (±8)**	**114 (±47)**	**36 (±15)**	**244 (±123)**	**51 (±29)**	**1.7 (±0.8)**
Fruits	IF	*A. caven*	329 (±156)	6.7 (±0.8)	0.3 (±0.1)	2.3 (±0.2)	2.4 (±1.4)	0.3 (±0.1)	0.2 (±0.1)	3 (±2)	3 (±1)	11 (±2)	4 (±2)	28 (±16)	6 (±2)	12 (±5.0)
	SF	*A. caven*	76 (±43)	1.5 (±0.8)	0.1 (±0.1)	0.5 (±0.2)	0.5 (±0.3)	0.1 (±0.1)	0.1 (±0.1)	4 (±3)	3 (±1)	11 (±4)	4 (±2)	29 (±13)	7 (±4)	3 (±2.0)
		P. affinis	30 (±23)	0.5 (±0.8)	0	0.2 (±0.3)	0.2 (±0.2)	0	0	9 (±8)	3 (±1)	23 (±12)	12 (±4)	25 (±11)	6 (±3)	1 (±1.0)
		C. ehrenbergiana	36 (±14)	0.9 (±0.8)	0.1 (±0.1)	0.2 (±0.1)	3.7 (±1.4)	0.1 (±0.1)	0	17 (±12)	6 (±3)	31 (±17)	26 (±14)	38 (±27)	11 (±5)	5 (±2.0)
		Total *SF*	**142 (±74)**	**2.9 (±2.5)**	**0.2 (±0.2)**	**0.9 (±0.7)**	**4.4 (±4.1)**	**0.2 (±0.2)**	**0.1 (±0.1)**	**30 (±23)**	**12 (±5)**	**65 (±32)**	**42 (±20)**	**92 (±49)**	**24 (±18)**	**9 (±3.0)**
	MF	*A. caven*	3 (±2)	0.1 (±0.8)	0	0	0	0	0	1 (±1)	1 (±1)	2 (±1)	1 (±1)	4 (±1)	1 (±1)	0.1 (±0.1)
		P. affinis	1 (±1)	0	0	0	0	0	0	3 (±2)	1 (±1)	6 (±2)	3 (±1)	7 (±3)	2 (±1)	0
		C. ehrenbergiana	39 (±26)	0.9 (±0.8)	0.1 (±0.1)	0.2 (±0.1)	4.1 (±2.2)	0.1 (±0.1)	0	25 (±12)	9 (±4)	46 (±22)	37 (±18)	55 (±26)	15 (±6)	6 (±2.0)
		P. nigra	24 (±11)	0.5 (±0.8)	0	0.2 (±0.2)	0.1 (±0.1)	0	0	23 (±23)	8 (±3)	41 (±19)	33 (±17)	49 (±26)	14 (±8)	1.0 (±0.3)
		Total *MF*	**67 (±58)**	**1.5 (±3.4)**	**0.1 (±0.1)**	**0.4 (±0.3)**	**4.2 (±2.3)**	**0.1 (±0.1)**	**0**	**52 (±37)**	**19 (±8)**	**95 (±48)**	**74 (±38)**	**115 (±57)**	**32 (±17)**	**7 (±3.0)**

The three forests studied differed significantly in terms of the contribution of leaf and branch fractions (the fractions of interest when characterizing forests in a succession); therefore, the L/B ratio has been used as an index of maturity in forests (Martín *et al*., 1993). The variation of L/B values is probably related to the dominance or development degree of each species in each forest. While leaf contribution often largely exceeds branch contribution in some temperate forest ecosystems (Gallardo *et al*., 1989; Drake *et al*., 2010), we found the opposite trend in the L/B ratio in the forests studied here in relation to the forest successional stage (mean values of 1.8, 1.5, and 0.8 for *IF*, *SF*, and *MF*, respectively, in the Mesopotamian Espinal). These results may be due to the fact that the species studied are xerophilous (with a low L/B ratio), with slow growth and higher wood retention in the plant.

In *MF*, *A. caven* was the species with higher branches contribution, with an L/B ratio of 0.3; however, values of 0.5 were recorded in *SF* and 1.8 in *IF*. *A. caven* is a colonizer species that may vary its proportion of branch production depending on the growing conditions and successional status. The species exhibited limited litterfall production and growth in *MF* due to the dominance of *P. nigra* and *C. ehrenbergiana*, which might be associated with a low photosynthetic biomass and high proportion of senescent branches. By contrast, in *IF* (colonized by *A. caven*) it had the lowest branch contribution (17%), mostly due to the lack of woody competitors (and probably higher availability of nutrients), which was then translated into active growth with greater photosynthetic biomass and lower proportion of senescent branches.

Likewise, *P. affinis* (which was dominant in *SF*) had an L/B ratio of 1.4 and 0.5 in *SF* and *MF*, respectively (Table 4); this was related to the maturity of the trees, where the ratio L/B tended to decrease in the forests with the most mature trees.

Maximum L/B values were detected in *C. ehrenbergiana* (2.6 in *SF* and 2.1 in *MF*, Table 4), especially in *SF*, indicating a more favorable competition with *P. affinis* than with *P. nigra* in *MF*.

The maximum contribution of fruits was recorded for *A. caven* in *IF*, (representing 29, 8, and 2 % in *IF*, *SF* and *MF*, respectively) might be explained by the capability to maximize fruit productivity to ensure a reproductive success and colonization of this species under adverse conditions (Duran & Kattan, 2005; Harvey *et al*., 2007).

Annual contribution by the fraction named 'other'

The contribution of this fraction is difficult of establish, by the majority of it could be associated with the presence/absence of shrub species, mainly of the genus *Baccharis*; a relatively high contribution of the "others" fraction (approximately 20 % of total production) can be expected in mixed forests (Aceñolaza *et al*., 2009), whose shrub layer has many species of the *Asteraceae* family.

The maximum contribution of the "others" fraction corresponded to *A. caven* in *SF*, *P. affinis* in *SF*, and *C. ehrenbergiana* in *MF*; whereas the minimum was recorded for both *A. caven* and *P. affinis* in *MF* (Table 4). Those results might be attributed to the effect of competition for light and nutrients among species of shrub and those appearing in the different vegetation layers (as in *MF*); this fact suggests a higher vulnerability to invasion by *A. caven* in *SF*, which has in *MF* lower cover than *P. nigra*.

Seasonal pattern of litter production

Regarding the relationship between seasonal litterfall production pattern and environmental variables, Aceñolaza *et al*. (2009) observed significant correlations between litterfall production and water level variation of the Paraná River in forests of its alluvial plain basin (eastern Argentina). Nevertheless, climatic variables, considered as conditioning factors (such as precipitation, wind, or temperature), did not influence significantly the seasonal pattern of litterfall in our study during the studied years.

The analysis of total contribution of species and forests per season allowed us to describe well defined patterns. A peak of contribution was observed in summer, which was significantly different from that of the remaining seasons ($p < 0.05$) for *A. caven* in *IF* and *MF*, and for *P. affinis* in *MF* (Table 3). This finding is in agreement with similar studies conducted in Mediterranean forests, where summer droughts can accelerate leaf abscission, conditioning the annual pattern of leaf production of deciduous oak (*Quercus pyrenaica*) forests. A similar situation was also observed for oak (*Q. rotundifolia*) forests (Santa Regina *et al*., 1997; Piao *et al*., 2006). The peak of production found in *A. caven* in *IF* in summer could indicate that this species was affected by seasonal water deficit. Maximum contribu-

tion in summer is associated greater evapotranspiration, increasing water stress; this result is in agreement with results reported by Santa Regina *et al*. (1997).

In agreement with Jeffrey *et al*. (2007), we observed that litter contribution is a result of complementary addition between *P. nigra* and *C. ehrenbergiana* in *MF*, and between *A. caven* and *C. ehrenbergiana* in *SF*. Overall, individual production of species is responsible for a constant contribution of organic residues throughout the annual cycle in *SF* and *MF*, although some significant differences among seasons were found.

These results show that maximum contribution in intermediate and advanced successional stages could be associated to the summer water deficit and species phenology characteristics (Mendoza *et al*., 2012).

PRB in the forest succession

As the chrono-sequence progressed, Ca replaced N in order of importance, due to the decrease of the leguminous species in the colonizing process after the *IF* stage, and the contribution to return of Ca by *C. erhenbergiana* in both *SF* and *MF*; then, the influence of the amount produced is much more important that the leaf concentration. The decline of Zn in *SF* and *MF* is noticeable, which is displaced by Mn, possibly because of presence of soil Ca (Table 1) and the limitation for plant absorption of Zn (Vogel *et al*., 2012) in *IF*. Another interesting process is the displacement of P by S in the *MF*; this fact indicates the importance of P availability in the first stages of the forest establishment.

PRB per species

In all three forests, *A. caven* showed the same order of importance of the elements found in the litterfall (Table 5).

According the order indicated for PRB in forests, N is the element of greatest potential return and shows the importance of the N-fixing capacity of the species of the family *Fabaceae* (Vogel *et al*., 2012).

The same order of importance of the bio-elements was observed for *P. affinis* in *SF* (Table 5), except that N was displaced by Ca, and P by S (under similar soil conditions; Table 1). As indicated above, this change in order could be associated with limitations imposed

by dominant species, which conditions N biological fixation and P uptake.

C. ehrenbergiana in both *SF* and *MF* showed maximum contribution of Ca (Table 5), which might be attributed to the presence of Ca carbonate phytoliths (Fernández *et al*., 2005) in leaves; therefore, leaf accumulation of cations means higher return.

P. nigra (Table 5) contributed with a maximum value of N in *MF*, which was consistent with its biological fixing capacity. Another important factor observed (Table 5) was the high potential return of S and P (displacing Mg), whereas the remaining elements showed the same order of importance described for *A. caven*. The higher potential returns of S and P might be attributed to a strong absorption of these elements and the limitations for the other species (Vogel *et al*., 2012), as observed in *P. affinis* in *MF*.

PRB considering the litterfall fractions

PRB values found considering the litterfall fractions showed that the leaf fraction was not important for all the species. As observed, leaf fractions have higher contributions on PRB of litterfall in some species than in others (Table 6).

Hence, here L/B ratio could also considered an index because it is related to the PRB values. The greatest leaf/total production ratio values were 0.55 and 0.52 in *C. ehrenbergiana* (*SF* and *MF*, respectively), which is consistent with production of a deciduous species. By contrast, *A. caven* (a semi-deciduous species) showed limited growth and development in *MF*, but only contributed with 15 % of leaves (Aceñolaza *et al*., 2010).

The highest PRB values pertaining to the leaf fraction of *C. ehrenbergiana* in *SF*; also a high value for branches was found in *P. nigra* in *MF* (Table 6). This shows complementarity not only in the temporal contribution pattern between those species, but also in the types of fractions and could be used as a strategy for bio-element return (Patrício *et al*., 2012). As mineralization in leaves is more rapid than in branches, bio-elements in leaf fraction of *C. ehrenbergiana* can be easily released, unlike those included in the branch fraction of *P. nigra* (that are released at a lower rate), with consequences in the nutrition of both species.

Based on our data and analyses, we conclude that:

— The productivity of the ecosystems of the Mesopotamian Espinal is rather low and becomes stable when the *SF* successional stage is established.

— Seasonal evolution of litterfall production in the forest successional sequence is determined by the production rate of the dominant species, decreasing in *IF*, in general, after summer, and showing a constant pattern in *SF*, and some sporadic significant differences in *MF*.

— The L/B ratio in forests seems to be a good indicator of the successional stage of semi-deciduous forests of the Mesopotamian Espinal; *i. e.*, the lower the L/B ratio, the higher the forest maturity.

— The contribution of flowers represents a very low percentage of the total annual litterfall production (below 3 %).

— The highest litterfall production by fruit is associated with a colonization strategy (as observed for *A. caven* in *IF*).

— Total PRB was proportional to litterfall production in the successional sequence studied, with maximum N values found when leguminous species are dominant; meanwhile *C. ehrenbergiana* shows the highest contributions of Ca.

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