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SOIL SCIENCE

Temporal Dynamics of Leaf Litter Components during Decomposition of an Aromatic Shrub *Cistus Monspeliensis* L.

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ABSTRACT

Litter decomposition in shrubland ecosystems is relatively not well understood, yet they cover significant proportion of the earth surface. The aim of the study is to understand the litter decomposition process of shrubland leaf litter under controlled conditions using litter bag technique over 360 days period. Destructive sampling was done at 0, 15, 30, 60, 90, 120, 150, 180, 270 and 360 days after the incubation and accumulated mass loss (AML) was calculated. We also examined release, retention or accumulation of the various nutrients, change in soluble compound, hemicellulose, cellulose and lignin concentrations in the residual litter. AML displayed a bi-phasic pattern with initial rapid phase, followed by a steady phase. AML best fitted to a double exponential decay model and decay constant for the rapid phase was approximately 130 times higher than the slower phase. Among the nutrients rapid release of P and K was observed. Soluble component concentrations decreased rapidly whereas hemicellulose concentrations slowly decreased. Conversely, lignin concentrations increased throughout the study; however cellulose concentrations are broadly stable. Litter AML best correlated to the temporal dynamics of Lignin-to-N ratio during the decomposition. Overall, 44% of the litter remain in the soil after 360 d of incubation period. Our results suggest that *Cistus* leaf litter contributes 8.3 kg $ha^{-1}d^{-1}$ C to below ground. However, details of *Cistus* species distribution and phenology need to be considered before these results are extrapolated to shrubland ecosystem.

Highlights

- Leaf litter decomposition study was conducted for 360 days period under controlled conditions using litter bag technique
- AML displayed a bi-phasic pattern with initial rapid phase followed by a steady phase, which is best correlated to the temporal dynamics of Ligni-to-N ratio in residual litter, contributing 8.3 kg ha^{-1} d^{-1} C to the below ground

Keywords: Leaf litter decomposition, C sequestration, Hemicellulose, Cellulose, Lignin, Ligni-to-N ratio

Soils are postulated as the largest C (Carbon) reservoirs on terrestrial ecosystem consisting of two times more C than the atmosphere and three times more C than vegetation that they support (Rustad *et al.* 2000). Therefore, exploring the mechanistic processes in relation to C fluxes such as litter decomposition is fundamental, whilst understanding soil C sequestration. However, litter decomposition in shrublands is relatively not well understood, yet they cover significant proportion of the earth surface (Incerti *et al.* 2011). Under arid environments such as the Mediterranean shrublands leaf shedding is a common phenomenon that occurs due to warm and dry climatic conditions, in addition to the plant natural phenology, thus contributing significant amounts of belowground C and nutrients (de Dato *et al.* 2013; Estiarte and Peñuelas 2015). Hence, understanding the leaf litter decomposition

process is essential in quantifying their contribution to soil organic matter and nutrients.

Litter decomposition is regulated by an array of factors such as climatic conditions, substrate quality, structure and size of soil biota. However, substrate quality is the primary controller of litter decomposition under a particular climatic condition. Substrate quality includes initial C, N, P concentrations (C-to-N or C-to-P ratio), cellulose, lignin content (lignin-to-N or lignin-to-P ratio), and nutrient concentrations (Mn, Ca etc.) in decomposing litter (Xiaogai *et al.* 2013; Paul *et al.* 2017). Litter N concentrations have been proposed to enhance the early decomposition rates (Melillo *et al.* 1982); conversely, it has a suppressing effect during later stages (Berg and Matzner, 1997). Plant litter consists of labile soluble components and relatively recalcitrant structural components such as hemicellulose, cellulose and lignin. The amount of C sequestered in the soil through litter decomposition is the function of litter amount diverted into humus through microbial and chemical reaction (Prescott, 2010). Lignin or acid unhydrolyzable residue (AUR) is the second most abundant plant synthesised compound after cellulose and contributes nearly 30% of the C sequestered in plant materials annually (Boerjan *et al.* 2003). Besides its recalcitrant nature, lignin is hypothesised to protect from the degradation of cellulose, hemicellulose, and protein in plant cell walls (Berg and Mc Claugherty, 2003). Conversely, among semiarid temperate grasslands lignin promotes the of plant litter decomposition due to photo degradation (Austin and Ballaré, 2010).

The aim of the study is to (a) quantify the litter contribution to soil C (b) investigate the changes in litter components during litter decomposition of a common aromatic shrub *Cistus monspeliensis* L. under controlled conditions using the litter bag technique.

MATERIALS AND METHODS

Soil characteristics

Soil used in this study was obtained from a temperate oceanic agricultural site at the Henfaes experimental station located in Abergwyngregyn, Gwynedd, North Wales (53° 14′ N, 4° 01′ W) UK. The sandy clay loam textured soil is classified as Eutric Cambisol (FAO) or Dystric Eutrudepts (US Soil Taxonomy). The mean annual soil temperature and annual rain fall are 11°C and 1250 mm respectively (Glanville *et al.* 2012). Soil was collected from Ah horizon (0-10 cm depth) and placed in gas permeable plastic bags and transferred to the laboratory (Venkata *et al.* 2017). Immediately after, soil was passed through a 2 mm sieve to remove stones, plant roots and earth worms. 10g of soil subsampled, dried overnight at 105 °C and the water content was calculated on the basis of weight loss. 5g of sieved soil subsampled in to 50 cm^3 polypropylene tube and 10ml of deionised water (1:2 w/v) was added, followed by shaking for 1 hour using an orbital shaker (250 rev min^{-1}). Soil pH and electric conductivity (EC) were measured. Samples were centrifuged (5000g) and supernatant was collected and analysed for available nitrates by vanadate method (Miranda *et al.* 2001), ammonium by salicylate-nitroprusside and hypochlorite procedure (Mulvaney *et al.* 1996) and free amino acids by fluorometric OPAME procedure (Jones *et al.* 2002). General properties of the soil presented in table 1.

Table 1: General properties of soil used in the present study. Values represent mean. Value presented in parenthesis indicates standard error mean; *n = 3*

Soil character	Measured quantity
Water content (%)	26.2 ± 0.1
pH (1:2 H_2O)	5.4 ± 0.1
EC (1:2 H_2O μScm^{-1})	169.4 ± 4.1
Available NO_3^- (mg N kg^{-1} soil)	10.2 ± 0.2
Available NH_4^+ (mg N kg^{-1} soil)	0.2 ± 0.1
Free amino acids (mg C kg^{-1} soil)	2.6 ± 0.9
Dissolved Organic C (mg kg^{-1} soil)	26.8 ± 4.3
Dissolved Organic N (mg kg^{-1} soil)	11.9 ± 2.5

Litter bags preparation, samplings and laboratory analysis

Senescent leaves fallen from the *Cistus* plants were collected and air dried at 80°C to a constant weight. 3g of dried leaves were placed into each litter bag of 8cm × 10cm size, made up of 1mm × 1mm aperture nylon mesh, which is wide enough to allow entry of microbes, microfauna and mesofauna but excludes macrofauna and prevents the loss of leaf fragments (Riutta *et al.* 2012). 30 litter bags filled with leaves were stitched at four sides to secure the leaf material and incubated one bag per pot of

11cm × 11cm size. Each pot was filled with 1000g of field moist soil and watered once a week to ensure natural moisture levels and the excess water is allowed to drain from the base of each pot. The pots were incubated under greenhouse conditions with temperatures maintained at 25 °C. Destructive sampling was done in triplicates after 0, 15, 30, 60, 90, 120, 150, 180, 270 and 360 d. Harvested samples were returned to the laboratory and litter bags were gently cleaned for adhering soil with a brush and cut opened. Leaf litter samples were air dried at 80°C to a constant weight, accumulated mass loss (AML) was calculated and expressed as % initial mass using the formula (Eq. 1) below:

$$\text{AML (\%)} = 100 \times (\text{initial mass} - \text{residual mass}) / \text{initial mass} \quad \ldots(1)$$

Leaf litter was analysed for soluble, hemicellulose, cellulose and lignin compounds at each sampling using an automated fibre analyser (Ankom Technology, USA). Litter weight loss during neutral detergent fibre (NDF), acid detergent fibre (ADF) and acid determined lignin (ADL) processes approximately represent soluble compounds, Hemicellulose and Cellulose fractions respectively. Subsequently, lignin concentrations were calculated by subtracting the ash content values (Table 2).

Table 2: Leaf litter analysis for various fractions (Source: Ankom protocol, ANKOM Technology, USA)

Extraction	Washed off fraction	Remaining fraction
NDF	Carbohydrates, lipids, pectin, starch, soluble proteins and non-protein nitrogen	Hemicellulose, proteins bound to cell walls etc. Cellulose, lignin and recalcitrant materials
ADF	Hemicellulose and proteins bound to cell walls	Cellulose, lignin and recalcitrant materials
ADL	Cellulose	Lignin and recalcitrant materials

Simultaneously, N quantity associated with soluble and structural components (initial litter only) was determined using TruSpec CHN analyser (Leco, USA) in remaining litter after NDF and ADF processes. Due to poor recovery of samples after ADL we did not determine the N bound to lignin fraction only.

Nutrient analysis

Sub-sampling was done to measure the nutrient content of the leaf litter to measure the changes during the leaf litter decomposition study period. Total carbon (C) and nitrogen (N) were determined with a TruSpec CN analyser (Leco Corp., St Joseph, MI, USA). For the remaining nutrients 20 mg of ground samples were placed in to micro centrifuge tubes, 1ml of Triton X100 solution (a sample suspension agent) was added. Further, 10µL of Ga (1000mg Ga in L^{-1} DI water) was added as an internal standard and mixed well for 20 seconds. Selected major nutrients *viz.*, phosphorous (P), potassium (K), calcium (Ca), and manganese (Mn) were determined using a total reflection X-ray florescence spectroscopy (S2 PICOFOX Bruker).

Data and statistical analysis

Leaf litter mass loss was best fitted to double exponential first order decay as given below (Eq. 2):

$$Y = W_1^{e-k_1 t} + W_2^{e-k_2 t} \quad \ldots(2)$$

Where Y represents the remaining litter mass after the incubation period t. W_1 and W_2 are labile and recalcitrant fractions of the leaf litter, whilst k_1, k_2 are the rate constants for W_1, W_2 fractions respectively. We have also calculated half-life period ($t_{1/2}$) which is time required to half the initial quantity of each W_1 and W_2 litter fractions using the following Eq. 3 where k represents (either k_1 for labile or k_2 for recalcitrant litter fractions) the decomposition constant.

$$t_{1/2} = 0.693/k \quad \ldots(3)$$

Lignin cellulose index (LCI) was calculated using the Eq. 4 at each sampling point (Herman *et al.* 2008).

$$\text{LCI} = (\text{lignin}) / (\text{lignin} + \text{hemicellulose} + \text{cellulose}) \quad \ldots(4)$$

Data for litter AML and nutrient dynamics were processed using Sigma plot v12.3 (Systas Software Inc., Chicago, IL) and the corresponding mean values were compared applying one way ANOVA with PostHoc Least Significant Difference (LSD) test using SPSS v20.0 (SPSS Inc., Chicago, IL). We accepted $P < 0.05$ as an indication of statistical significance.

RESULTS AND DISCUSSION

Accumulated mass loss (AML)

Litter AML displayed a biphasic pattern, an initial rapid phase followed by slower phase (Fig 1A). During the first 15 days AML was 23% and subsequently reached 50.3% within 90 days after incubation. However, at the end of 360 day study period 56.7 % of AML was observed. Modelled results showed that half of the litter mass was allotted to rapid decomposition phase (W_1) and the decay constant (k_1) values were *ca.*130 times faster than the slower phase (W_2). Consequently, half-life periods of W_1 and W_2 substrate pools varied substantially (Table 3).

Table 3: Modelled double exponential decay parameters. W_1 represents the labile and W_2 recalcitrant pools (% initial mass) of the leaf litter material, k_1, k_2 are decay constants (g day^{-1}) and $t_{1/2a}$, $t_{1/2b}$ are the half- life period in days of the w_1 and w_2 respectively. Values represent mean, ± indicates standard error mean; n = 3.

W_1	k_1	$t_{1/2a}$	W_2	k_2	$t_{1/2b}$
53.5 ± 3.2	0.03	27.9 ± 4.5	44.8 ± 3.2	0.0002	2970 ± 810.3

Soluble components contributed *ca.*61.2% of litter mass at the beginning, but declined rapidly during the initial 90 days of decomposition reaching to 37.8% of residual litter mass (Fig 1B).

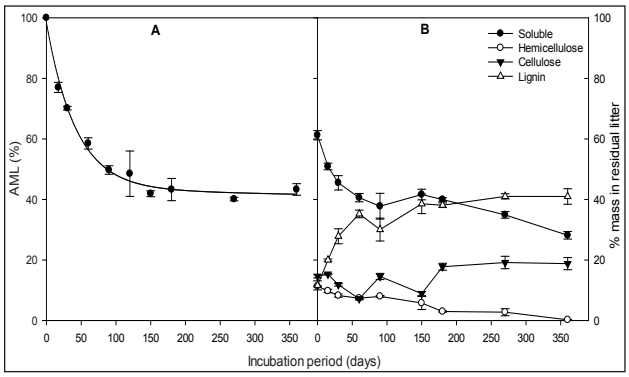

Fig. 1: AML during decomposition of a shrubland leaf litter (A). Changes in various component concentrations of leaf litter (B). Data represents mean. Error bars indicate standard error mean; n = 3

Further, concentrations declined only *ca.* 9% in next 270 days, reaching to 28.2% of residual litter mass at the end of study period. In contrast, hemicellulose content declined gradually from 11.4% to 0.3% of litter mass throughout the study period. Cellulose concentrations declined during the first 60 days (from 14.6% to 7.2%), later the values fluctuated and reached to 17.8 % at the end of 180 days period, and remained at similar levels until the end of the study. Lignin concentrations were increased for first 60 days (11.6 to 35.2%), but later remained stable until the end of the study period reaching to 40.1 % of the residual litter mass.

Similarly, LCI values increased for the first 60 days, but later remained broadly stable. Among litter components N was mostly distributed in the soluble fractions (*ca.*79%), whereas only ca. 21% total N bound to the structural components. Among the structural components *ca.*15% total N associated with hemicellulose and remaining N bound to cellulose and lignin together (Table 4).

Table 4: N distribution among various litter components. Values are means ± indicates standard error mean; $n = 3$

Litter component	% of total leaf litter N
Soluble	79.5 ± 11.5
Hemicellulose	15.8 ± 0.5
Cellulose + Lignin	8.8 ± 0.3

Nutrients in leaf litter

There was an initial small net accumulation in the N concentrations was observed for first 60 days and a subsequent release followed by a steady phase (Fig 2A). As expected, rapid release of leaf litter P was observed at the end of the first 15 days followed by a steady phase for 60 days and a further gradual release up to 120 days with no further significant change until end of the study period (Fig 2B).

Similarly, there was a rapid release of K for initial 15 days followed by a gradual release till the end of incubation period (Fig 2C). Ca concentrations in litter during first 15 days remain stable, but increased to 15.93 mg g^{-1}after 60 d and later decreased. Further, Ca concentration increased at 270 days reached up to 225% of the initial concentrations by the end of the study (Fig 2D). Mn concentrations have fluctuated throughout the study period (Fig 2E).

The main emphasis of our study was to quantifying the litter contribution to the below ground C, whilst elucidating the changes to litter components during the decomposition process.

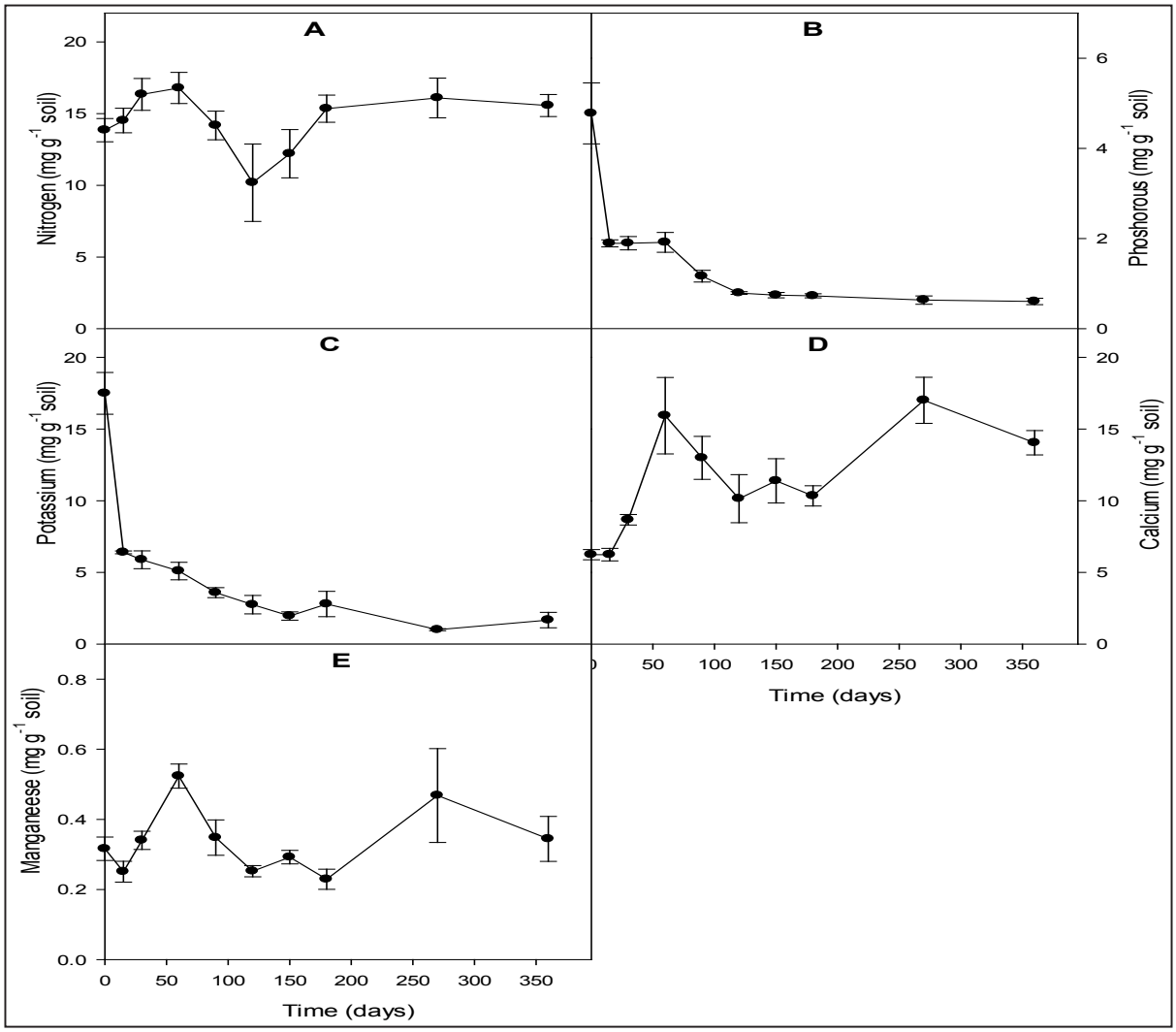

Fig. 2: Temporal dynamics of Leaf litter nutrients during decomposition. (Data represents mean. Error bars indicate standard error mean; *n* =3. (A) N (B) P (C) K (D) Ca (E) Mn

Litter contribution to soil C

AML occurred in a biphasic manner and data best fitted a double exponential model as reported earlier for C. *monspeliensis* L. leaf litter (Gillon *et al.* 1994). This indicates that the litter substrate has two substrate quality pools with different decomposition rates *viz*., labile (W_1) and relatively recalcitrant (W_2) pools. The initial loss is mainly attributed to the release of water soluble and non-structural compounds in the leaf litter (Prescott, 2005). The released water soluble compounds are labile in nature and may have either mineralised or incorporated into the soil microbial biomass (Venkata *et al.* 2016). Our results contradicts with Saura-Mas *et al.* (2012) where only *ca.* 11% and 42% of initial mass loss was observed after 2 and 24 months of incubation period respectively under Mediterranean climatic conditions, whilst here we noticed 41.48% mass loss within first 2 months. Moreover, data best fitted to a single exponential decay model in that study. This was presumably due to a) variation in initial litter chemistry, for example initial litter total N content (Berg and Matzner 1997); b) soil physio-chemical properties; c) soil microbial size and structure; d) mean annual temperatures and precipitation.

Overall, *ca.* 44% of litter biomass remains in the soil after 360 days of incubation period. *C. monspeliensis* L. is a semi deciduous shrub, adds approximately 42 kg m^{-2} d^{-1} leaf litter during early summer under Mediterranean climatic conditions (de Dato *et al.* 2013).Therefore, based on results from the present

study, 18.5 Kg.m^{-2} d^{-1} (*ca.* 44%) litter residue remains in the soil after 360days. Given the fact that leaves consist of *ca.* 45% of their total biomass as C (Venkata S S R Marella 2015), we estimate that during early summer (litter fall period) ca. 8.3 kg ha^{-1}d^{-1}C will have been added to the soil (Berg and McClaugherty 2014). However, microbial incorporation of labile compounds through various metabolic pathways need to be accounted to give more precise value of litter contribution to stable organic matter (Cotrufo *et al.* 2015). Further, distribution of *Cistus* species and their phenology need to be considered before these results are extrapolated to the shrubland ecosystem. Nevertheless, these values emphases the leaf litter contribution to an under researched ecosystem.

Changes to litter components during decomposition

Soluble and structural components contribute *ca.* 60 and 40 % of the litter biomass. Structural compounds such as hemicellulose, cellulose and lignin need to be depolymerised into simpler compounds for the microbial uptake (Paul 2007). Soluble compounds and hemicellulose concentrations declined from the beginning of the study period, which contributed to the overall mass loss (Fig 1B). This also implies that lignin has weakly protected hemicellulose from the decomposing organisms (Talbot and Treseder 2012). Conversely, cellulose concentrations were broadly constant throughout the study period and a similar trend was reported earlier despite the variation in initial cellulose concentrations (Fioretto *et al.* 2005). Lignin concentrations in residual litter have increased for initial 90 days probably due to faster release of other components (soluble and hemicellulose) and inclusion of humic substances produced during the decomposition (Coûteaux *et al.* 1996). Consequently, the measured lignin concentrations in our study reflect the native lignin and acid unhydrolyzable residue (AUR) that are sourced through the microorganisms during the

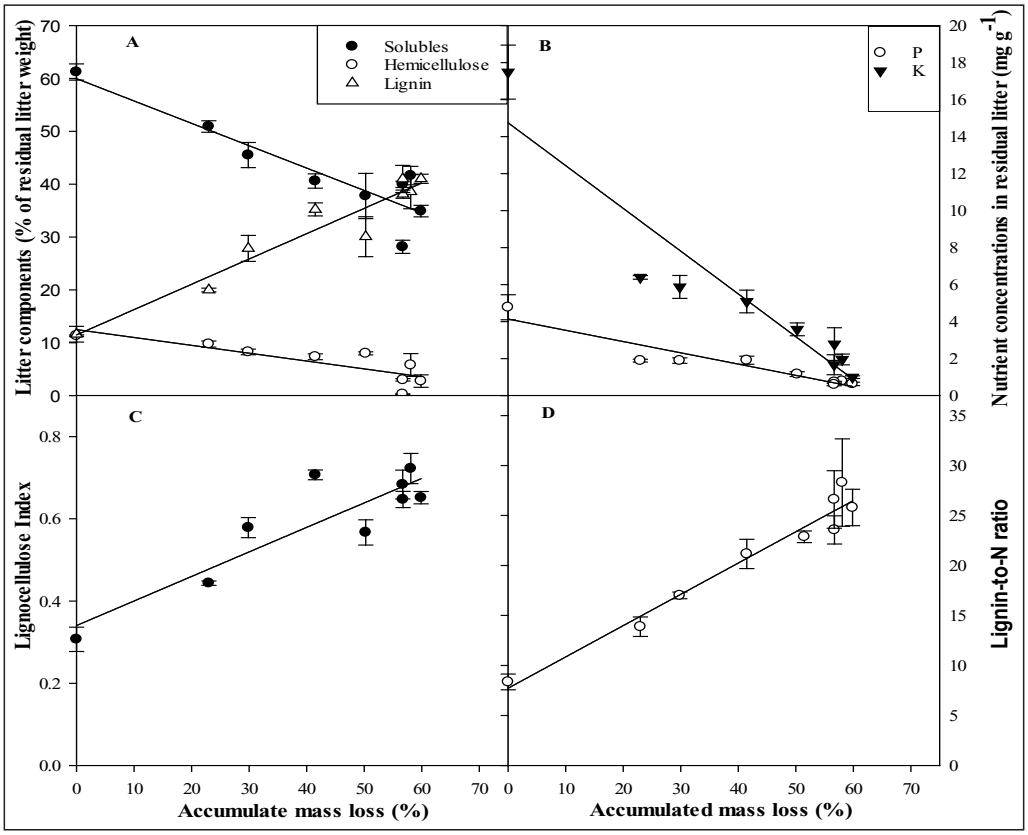

Fig. 3: AML (%) relationship with litter chemistry (A) various litter components (%). Soluble compounds (-ve, r^2=0.82; $p < 0.001$); Hemicellulose (-ve, r^2=0.70; $p = 0.005$); Lignin (+ve, r^2= 0.92; $p < 0.001$) (B) selected major litter nutrient concentrations P (-ve, r^2=0.90; p =0.001); K (-ve, r^2=0.89; p=0.001) (C) LCI (+ve, r^2= 0.79; $p = 0.001$) (D) Lignin/N (+ve, r^2= 0.96; $p < 0.001$). Error bars represent standard errors mean; *n=3*

decomposition process (Fioretto *et al.* 2005). Our study results are supported by the argument that litter with low initial lignin content (< 30%) show an absolute increase before net decomposition of lignin begins (Rutiglino *et al.* 1996). Moreover, the amount of N bound to cellulose and lignin together was very low (*ca.* 8.81%) giving less incentive to the microbes to decompose; hence we did not see decrease in lignin concentrations during incubation period. Similar results were reported by Fioretto *et al.* (2005), where lignin decomposition delayed due to less N is bound to lignin. The release of hemicellulose during the initial stages coupled with the accumulation of lignin has clearly reflected in lignin cellulose index (LCI) values (Fig 3C). However, despite LCI values reaching 0.7, we did not see lignin release (Herman *et al.* 2008), presumably because of more N bound to litter labile fraction and/or lower Mn concentrations in litter may have suppressed the lignin degradation enzymes (Berg, 2014b). Overall, among all the measured substrate parameters litter lignin-to-N ratio dynamics best correlated to the AML (Fig 3 A, B, C, D).

Nutrient dynamics

In the present study, temporal dynamics of each nutrient have varied substantially compared to previous studies. Initial increase in litter N concentrations was presumably due to microbial immobilisation. However, leaching may have counter balanced the N accumulation therefore only a small amount of N immobilisation was observed, although we did not measure the quantity and quality of leachates (Zeller *et al.* 2000). Initial litter P concentrations are significantly higher compare to the values in earlier studies, consequently, C-to-P ratio values were substantially lower (Gillon *et al.* 1994; Saura-Mas *et al.* 2012). As expected, the release of P has occurred since litter initial C-to-P ratio was well below the proposed threshold value (< 700) for P release (Moore *et al.* 2006). N-to-P ratio increased during the entire study period despite the release of N between 60-120 d after incubation indicates that faster P release than N. The rapid release of K was mainly because it is a non-structural component with higher solubility (Li-Xin *et al.* 2003). Among the nutrients K had the most rapid release rate followed by P. The temporal dynamics of N, P and K were broadly similar to that of observed by Saura-Mas *et al.* (2012) among Mediterranean basin-woody species. The observed increase in Ca concentrations was probably due to the faster release of labile components, since, Ca is primarily associated with structural components.

CONCLUSION

In conclusion our results demonstrate that leaf litter contributes significant amounts of soil C in shrubland ecosystem. Soluble components and hemicellulose are the major contributors to the litter AML. Among the litter nutrients K had the most rapid release rate followed by P. Litter chemistry altered substantially during the decomposition process. However, lignin-to-N dynamics during decomposition best correlated to the litter AML. Our research results can also be applied in sustainable agriculture production whilst synchronizing the litter nutrient release pattern with targeted crop requirement using natural or artificial methods (Game *et al.* 2016).

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