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Decomposition and nutrient release pattern of three potential leguminous green manure crops of Mizoram

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Abstract

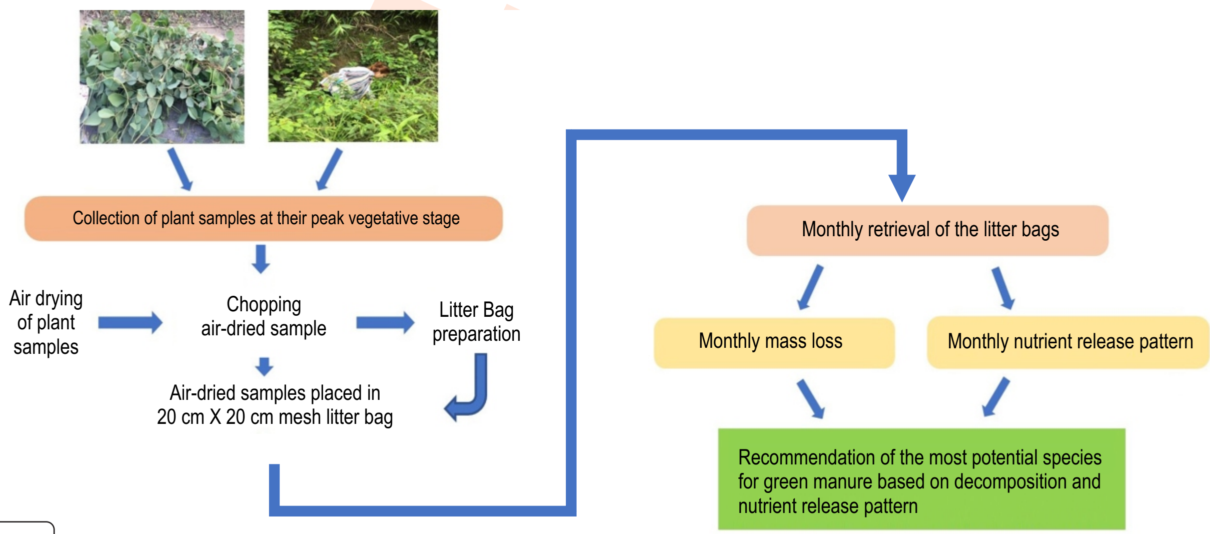
Aim: The experiment was carried out to understand the decomposition and nutrient release dynamics (N, P and K) of *Crotolaria micans* Link, *Aeschynomene indica* L. and *Calopogonium mucunoides* Desv. using the litter bag technique.

Methodology: The species were harvested at their peak vegetative stage and air-dried for a week. Air-dried samples (10 g) were placed in 20 cm X 20 cm nylon mesh bags and buried at 10 cm soil depth in an experimental plot of 1.5 m X 1.5 m prepared for each species. The bags were retrieved monthly, and the mass and nutrient loss were determined.

Results: *C. micans* had the highest decay rate (7.67 *k* per year), followed by *C. mucunoides* (7.15 *k* per year) and *A. indica* (5.50 *k* per year). During the decomposition period, K release was the fastest. The decay rate was negatively correlated with initial lignin, total phenol and lignin/N of which the total phenol (R^2 = 0.956) and lignin/N ratio (R^2 = 0.832) had the most significant influence on decomposition.

Interpretation: The study reveals that the species can be effectively utilized as a soil nutrient input source of which *C. micans* seems to be the most potential species to be further researched and developed as a green manuring crop.

Key words: Decomposition, Decay rate, Green manure, Legume, Nutrient release



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Introduction

Over 50% of net primary production in terrestrial environments is recycled into the soil by decomposition (Wardle *et al.,* 2004). Decomposition is a process that returns nutrients and organic matter from plant vegetative parts to the soil. It includes physiochemical and biological processes where the organic matter is transformed into increasingly fine forms that act as a source the plants utilize for their growth (Hasanuzzaman and Hossain, 2014). The primary elements governing the litter decomposition are the local climate, the quality of plant litter, and the type and amount of decomposing organisms (Garcia-Palacios *et al.,* 2016). Three major elements that govern the process of decomposition and nutrient release from organic residues applied to soil in agricultural and natural systems are the physical-chemical conditions of the environment, which are influenced by climate and soil properties; the type of vegetation that determines the quality and degradability of organic substances; and the nature of soil micro-organisms and soil fauna (Mangaravite *et al.,* 2023).

The initial substrate quality of litter such as nitrogen and lignin concentrations influence the litter decay pattern (Deb *et al.,* 2005). Plant material with higher concentration of lignin retards the rate of decomposition and nutrient release pattern and also the polyphenolics content in the plant also lower the rate of decomposition and nitrogen release by binding to nitrogen-containing compounds in the plant material (Abay *et al.,* 2015). High breakdown rates and nutrient release are associated with high nutrient levels in plant materials, which promote microbial activity and growth (Cobo *et al.,* 2002). The amount of structural components like cellulose, lignin and polyphenols, as well as the ratios of C/N, C/P, polyphenol/N and lignin/N define the relative significance of nutritional contents (Lupwayi *et al.,* 2004). Decomposed plant biomass can be an excellent source of organic material for soil health and crop growth. Legumes used as green manure have been an alternative for agricultural regions by providing essential nutrients to the soil through their biomass decomposition (Lalremsang *et al.,* 2022).

Green manure decomposition and the following release of N mainly depends on the quality and quantity of residue and soil factors like moisture, temperature, texture, biological activities and the presence of other nutrients in the soil (Thonnissen *et al.,* 2000). Mizoram, a hilly state located in the north-east region of India is rich in biodiversity and harbors many plant species, which are otherwise considered weeds although may have the potential to be utilized as green manure crops. However, the potential value of green manures as a source of nutrients for crops can only be realized if their patterns of nutrient release and decomposition are comprehended, allowing for an increase in crop nutrient demand (Cobo *et al.,* 2002). Therefore, the aim of this study was to determine the decomposition and nutrient release pattern of three abundantly available leguminous plants of Mizoram to assess their potential to be utilized as green manure crops.

Materials and Methods

The study was carried out in an experimental field in the year 2020, at Mizoram University. Aizawl, India. The soil of the study area (at 10 cm depth) was sandy loam in texture and acidic in nature (5.4 pH). The annual rainfall during the year of study was 167.4 cm with a mean temperature of 22.33°C. The maximum temperature occured in August while the minimum in January (Fig. 1). The monthly data for rainfall, temperature and relative humidity were collected from the Directorate of Science and Technology, Government of Mizoram.

Plant sample collection, preparation and decomposition study: Fresh plant samples of *Crotolaria micans* Link, *Aeschynomene indica* L., and *Calopogonium mucunoides* Desv. were collected from the wild at their peak vegetative stage, chopped into small pieces and air-dried. The litterbag technique (Bocock and Gilbert, 1957) was used to examine the decomposition rates of three species. Air-dried samples (10g) were placed in 20 cm X 20 cm nylon mesh bags having 1 mm X 1 mm mesh size. For each species, a 1.5 m X 1.5 m plot size was prepared in a total experimental field area of 22 m X 8 m. The plots were replicated 5 times for each species (3 treatments), thus making a total of 15 plots, and 15 litter bags per plot for each species was buried at 10 cm soil depth. One bag from each plot was retrieved at monthly intervals. Each time, the bags were removed, clinging residual materials were thoroughly brushed and rinsed to eliminate soil and other extraneous material, and the remaining material was oven dried at 600°C until constant weight was reached before being weighed to calculate the mass loss. The oven-dried samples were sieved and ground into powder for chemical examination. Dry mass from five litter bags per species was pooled together for chemical analysis.

Chemical analysis: Carbon and Nitrogen contents were determined with a CHNS analyzer (Elementar vario Marco cube). Total phosphorous content was estimated by diacid digestion method measured and using a spectrophotometer calorimetrically. The cellulose and lignin content were estimated by the Van Soest method (Van Soest *et al.,* 1991). Potassium content was determined by Microwave Plasma-Atomic Emission Spectroscopy (Agilent Technologies 4100). Total phenol content in the plant samples was carried out by Folin- Ciocalteu reagent (Bray and Thorpe, 1954).

Computation and statistical analyses: The decay constant of the species was calculated by negative exponential decay model (Olson, 1963): $X/X_0 = \exp^{(-kt)}$ or $k = -\ln(X/X_0)/t$, where, X is the weight remaining at t time, X_0 is the initial weight; t is the time in a year and k is the decay rate constant. The time required to achieve 50% (t_{50}) and 99% (t_{99}) decay was calculated as $t_{50} = 0.693/k$ and $t_{99} = 5/k$. The nutrient content of the decomposing species was derived by the formula: $(C/C_0) \times (DM/DM_0) \times 100$, where C is the concentration of nutrients at the time of sampling; C_0 is the initial nutrient concentration, DM is the mass of litter at the time of sampling, DM_0 is the initial mass (Bockheim *et al.,* 1991). One-way ANOVA was performed to compare the means of initial litter chemistry and decay rate of three selected species using IBM-SPSS statistics, version 25. Linear regression function was carried out between the decay rate and the initial chemical parameters of the plant materials.

Results and Discussion

The initial composition of the test (leguminous) plants showed significant variation among them (Table 1). Nitrogen content was highest in *A. indica* (3.11 %) followed by *C. micans* (3.05 %), while the lowest amount of nitrogen was found in *C. mucunoides* (1.96 %). Phosphorous content was the highest in *C. micans* (0.27 %) and lowest in *C. mucunoides* (0.17 %). Total carbon content was found to be in order *C. micans* (38.12 %) > *A. indica* (35.5 %) > *C. mucunoides* (33.67 %). *C. micans* recorded the highest potassium content (1.55 %) among the studied species. Lignin was recorded maximum in A. indica (16.98 %) whereas the minimum was obtained from *C. mucunoides* (7.56 %). Cellulose was recorded maximum in *C. mucunoides* (32.19 %) and the least was obtained from *C. micans* (18.39%) respectively. The total phenolic content was recorded as the highest in *A. indica* (1.8mg/100g).

The lignin/ N ratio ranged from 3.86 to 5.45 in the axles *C. mucunoides* < *C. micans* and < *A.indica,* while, the C/N ratio ranged from 11.41 to 17.22 in the order *A. indica* < *C. micans* < *C. mucunoides.* The decomposition pattern differed considerably for all three legume species (Fig. 2). *C. micans* lost about 45% of the initial mass in the first month (30 days). About 90% of the initial mass was lost in the third month followed by a relatively slow phase of decomposition until the last incubation period. In *C. mucunoides*, about 38 % of the initial mass was lost in the first month of the decomposition period. About 90 % of the initial mass was lost in the fifth month (Fig. 2). At the end of the study period approximately 3% of the initial mass was retrieved from the litter bag. *A. indica*, followed the same decomposition pattern like other two species where decomposition was more rapid in the initial stage and retarted at later stages. In the initial stage, about 45 % of the mass was lost. About 7 % of the initial mass remained in the last month of the incubation period. The decomposition process is, however, thought to involve a quick leaching phase at first followed by a gradual period where soil microbial activity becomes preponderant (Mubarak *et al.,* 2008; Das and Mondal, 2016).

Other studies have also reported a faster rate of decomposition in the initial stages due to the presence of sugars, amino acids and proteins which are considered fast decomposable components and while in the later phases, the decay rate constant slows down as recalcitrant substances like lignin, tannins and cellulose build up (Lupwayi *et al.,* 2004; Thonnissen *et al.,* 2000). The decomposition rate of *C. micans* was the fastest among all the species considered in the study, closely followed by *C. mucunoides.* The decay rate constant varied from 5.50 to 7.67 k per year with *C. micans* having the highest followed by *C. mucunoides* and *A. indica* (Table 2). However, a study by Mangaravite *et al.* (2023) reported the decomposition constant (k) in the order: jack bean > dwarf Mucuna > pigeon pea > Sunhemp. The maximum days required

Table 1: Initial composition of leguminous plants

Components (%)	*Crotolaria micans*	*Calopogonium mucunoides*	*Aeschynomene indica*
Nitrogen	$3.04^{b} \pm 0.01$	$1.95^{c} \pm 0.01$	$3.11^{a} \pm 0.00$
Phosphorus	$0.27^{a} \pm 0.00$	$0.17^{b} \pm 0.00$	$0.25^{a} \pm 0.00$
Potassium	$1.55^{a} \pm 0.00$	$0.88^{c} \pm 0.00$	$1.46^{b} \pm 0.00$
Carbon	$38.12^{a} \pm 0.00$	$33.67^{c} \pm 0.01$	$35.50^{b} \pm 0.01$
Lignin	$12.71^{b} \pm 0.01$	$7.55^{c} \pm 0.04$	$16.98^{a} \pm 0.00$
Cellulose	$18.39^{c} \pm 0.21$	$32.19^{a} \pm 0.01$	$20.22^{b} \pm 0.01$
Total phenol (mg $100g^{-1}$)	$0.68^{b} \pm 0.01$	$0.73^{b} \pm 0.01$	$1.80^{a} \pm 0.03$
Lignin/N ratio	$4.17^{b} \pm 0.01$	$3.86^{c} \pm 0.01$	$5.45^{a} \pm 0.00$
C/N ratio	$12.52^{b} \pm 0.03$	$17.22^{a} \pm 0.06$	$11.41^{c} \pm 0.01$

Values are mean of five replicates ± S.E. that shares same alphabet along the rows are not significantly different ($p < 0.05$).

Table 2: Decay constant rate (k yr^{-1}) and the time required for 50 % and 99 % mass loss of three leguminous plants

Species	Decay constant	t_{50} (days)	t_{99} (days)
Crotolaria micans	7.67^{a}	32.99^{c}	238.04^{c}
Calopogonium mucunoides	7.15^{b}	35.60^{b}	255.43^{b}
Aeschynomene indica	5.50^{c}	46.17^{a}	331.86^{a}

Values are mean of five replicates, that shares same alphabets along the columns are not significantly different ($p < 0.05$)

Table 3: Relationship between the initial composition (X) and the leguminous plants decay rate constant (Y)

Litter composition	Regression equation	R^2	*p*
Nitrogen	Y = 8.344 – 0.581 X	0.111	NS
Phosphorus	Y = 7.258 – 2.105 X	0.11	NS
Potassium	Y = 7.426 – 0.505 X	0.026	NS
Carbon	Y = 0.837 + 0.166 X	0.107	NS
Lignin	Y = 8.821 – 0.165 X	0.472	0.05
Total phenol	Y = 8.653 – 1.764 X	0.956	0.05
Lignin/N	Y = 12.288 – 1.227 X	0.832	0.05
C/N	Y = 4.497 + 0.007 X	0.203	NS
Cellulose	Y = 6.177 + 0.001 X	0.083	NS

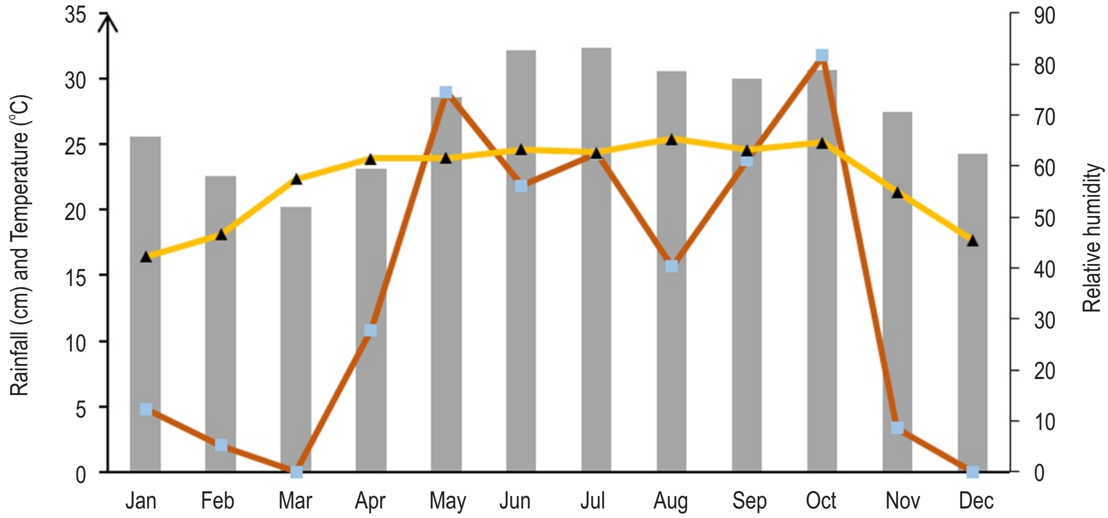

Fig. 1: Climatograph of study area where yellow line represents temperature; brown line represents rainfall (cm) and the bar chart represents relative humidity of 2020.

to decompose 50% and 99 % of litter was taken by *A. indica* and the minimum by *C. micans*. Similarly, a study by Upadhyaya *et al.* (2012) revealed that the *Citrus* sp. with the highest decay rate k value (8.16 $year^{-1}$) decomposed more rapidly among the 5 home garden tree species. The k values observed in the Indian Himalayan forests ranged from 0.006 to 4.71 $years^{-1}$ (Ahirwal *et al.,* 2021). Matos *et al.* (2011) reported the decay rate of four leguminous green manure plants in two different farms in the order *Calopogonium mucunoides* (0.004 day^{-1})< *Stizolobium aterrimum* (0.005 day^{-1}) < *Stylosanthes guianensis* (0.006 day^{-1}) < *Arachis pintoi* (0.007 day^{-1}). The decomposition rate was negatively correlated with lignin, total phenol and Lignin/N at ($p <$ 0.05), of which total phenol (R^2= 0.956) and Lignin/N ratio (R^2= 0.832) was considered to be the most influential in decomposition (Table 3). However, nitrogen, phosphorous, carbon, potassium, cellulose and C/N did not significantly correlate with the decomposition rate. The change in the decay rate can be attributed to the quality of the plant material and the soil organisms acting on it because the environmental conditions were same for all species. Lignin content in leaves has long been used to gauge for the quality of organic matter, where litter with lower lignin concentration favors the degradation process rather than higher lignin content (Lalremsang *et al.,* 2022).

The pace of decomposition may be explained by the lignin content of leaves alone or lignin/N (Butenschoen *et al.,* 2014). Our study revealed a negative correlation between the decay rate and lignin and Lignin/N which were considered to be the best predictors for litter decomposition rates, which also conforms to the report by Isaac and Nair (2006); Upadhyaya *et al.* (2012); Nath and Das (2011); Lalremsang *et al.* (2022). In this study, the lignin content in *C. micans* (12.71%) and *C. mucunoides* (7.56 %) was lower than *A. indica* (16.98 %) thus, promoting their faster rate of decomposition. The total phenol contents in the plant material also serve as an indicator of changes in the quality of litter, because they prevent microbial

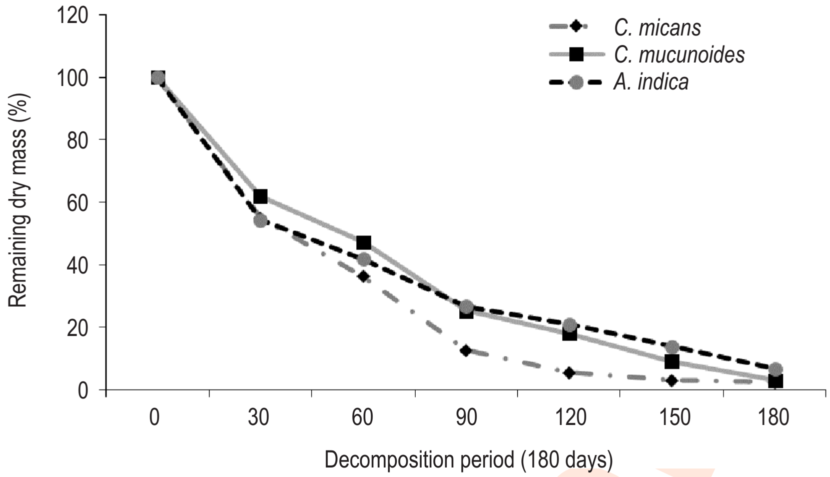

Fig. 2: Remaining dry mass remaining (%) in three leguminous weed species during decomposition period (180 days).

activity and precipitate protein from the litter (Palm and Sanchez 1990). The phenolic contents of *C. micans* was lower as compared to *C. mucunoides* and *A. indica* (Table 1) which may be the reason for slow decomposition of *A. indica* compared to the other two species studied. The phenolic content play a significant role in the degradation of biomass and the availability of nitrogen for soil microbes due to their ability to compound nitrogen quickly (Hattenschwiler and Vitousek, 2000)

The initial composition of the plant like the nitrogen content and C/N ratios, were also found to be a prominent factor in governing the plants' decomposition rate (Garcia Palacious *et al.,* 2013). The fastest breakdown rate was seen in litter with higher nitrogen and lower C/N ratios when compared to litters with lower levels of nitrogen and a greater C/N ratio (Nath and Das, 2011). Pereira *et al.* (2016) reported high C/N ratio (17.6) in *Crotolaria juncea* as the reason for its slow decay rate compared to *Cajanus cajan*, *Crotalaria spectabilis, Dolichos lablab, Canavalia ensiformes* and *Mucuna deeringiana*. *A. indica* in this study had the lowest decay rate although it had the highest nitrogen content compared to other two legumes. The lower decay rate of the species might be due to higher lignin, lignin/P and phenolic content. Similar study showed that although the nitrogen content was higher, Sharma *et al.* (2018) reported lignin as the limiting factor for lower decay rate of Perilla leaf litter.

During decomposition, nutrient release from the decomposing plant species varied considerably. The remaining nitrogen, phosphorus and potassium percent in the plant litter reduced after decomposition, with potassium showing more pronounced changes (Fig. 3. c). *C. micans* and *A. indica* followed the nutrient release (K > P > N) pattern during the decomposition period whereas *C. mucunoides* followed the pattern (K > N > P). A similar nutrient release pattern (K > N > P) from the litter of agroforest tree species was reported by Hasanuzzam and Hossain (2014). The proportion of liberated nutrients was highest for potassium in all the species after 180 days of decomposition, (99.6 % for *C. micans,* 99 % for *C. mucunoides* and 98.6 % for *A. indica*). At the end of the decomposition period 0.34 % (*C. micans*), 1.07 % (*C. mucunoides*) and 1.32 % (*A. indica*) of potassium remained in the plant residues. This type of release pattern has been reported in previous studies where K had the highest release rate (Lalremsang *et al.,* 2022; Das and Mondal 2016; Brunetto *et al.,* 2011; Gomez-Munoz *et al.,* 2014). Other investigations conducted in diverse soil and climatic conditions also found the K release to be high from biomass in various cover crops of Fabaceae (Gama-rodriques *et al.,* 2007; Pereira *et al.,* 2016). The rapid release in the K concentration during the decomposition period might be due to high mobility and leaching of the water-soluble form of nutrients (Jeong *et al.,* 2015; Patricio *et al.,* 2012). Moreover, the potassium is the most common ion in plant cells and its rapid rate of release can be related to the fact that it is present in ionic form, not connected to any structural component of plant tissue (Mangaravite *et al.,* 2023). The fast release of potassium into the soil shows that the period of adoption of agricultural crops in succession to green manure should be minimized to reduce potassium loss attempting to increase their use by future crops.

The study revealed that the release of nitrogen from three legumes started during the incubation period's initial phase, which may be accredited to leaching. The remaining percent of nitrogen in plant residues of the selected species at the end of the study period were 1%, 2.23% and 3.06% for *C. micans, C. mucunoides* and *A. indica,* respectively (Fig. 3 a). The decline in nitrogen concentration during the final stages of decomposition can be due to the increased demand for nitrogen caused by strong microbial activity (Lalremsang *et al.* 2022). The chemical makeup of different forms of litter may directly influence the rapid nitrogen release or immobilization of nitrogen (Semwal *et al.,*

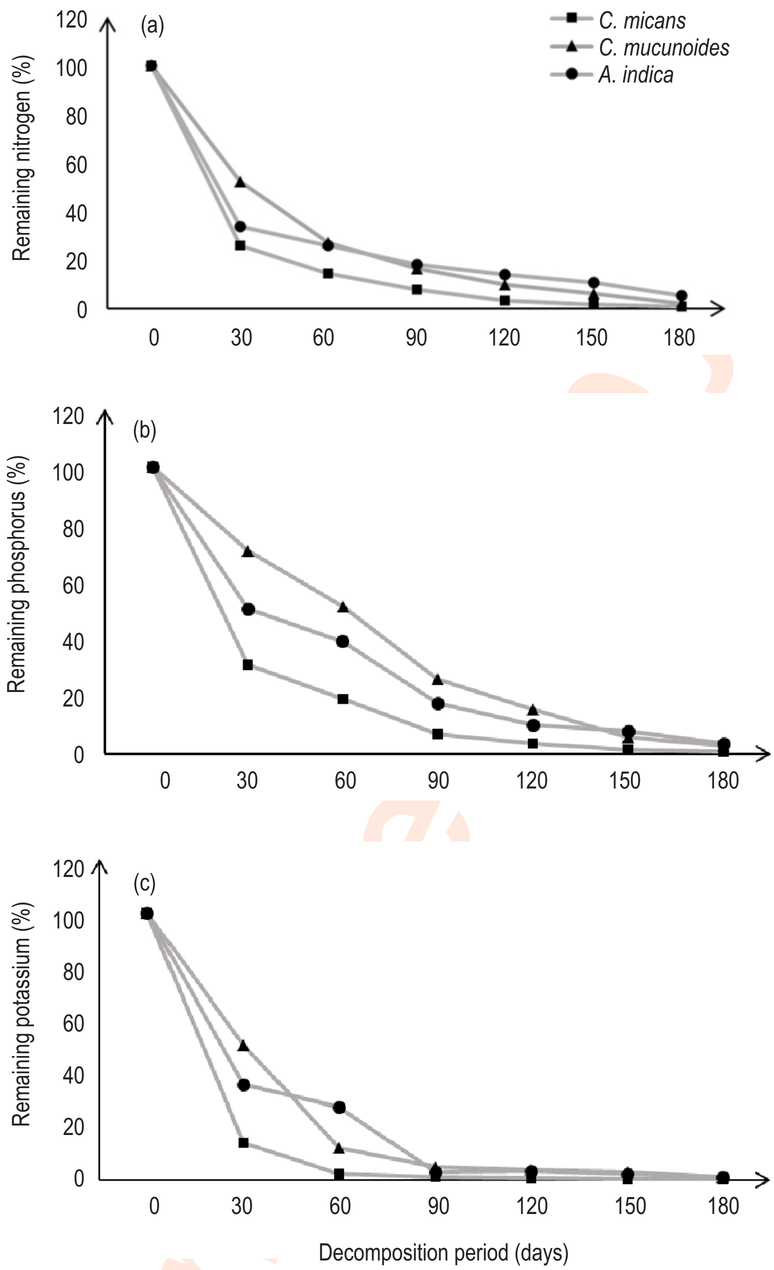

Fig. 3: Remaining nutrients (a: nitrogen; b: phosphorus and c: potassium) in leguminous plants.

2003). The amount of lignin in leaf litter is also crucial for the mineralization of nitrogen, leaf litter with less lignin decomposes more quickly and releases nitrogen at a faster rate as a result. The C/N ratio indicates the quality of the litter; it is said that high-quality litter has a C/N ratio (< 25) and releases nitrogen more quickly than litter with a C/N ratio greater than 25 (Myers *et al.,* 1994).

Legumes typically have lesser C/N ratios than other tree species, and lower C/N ratios are usually linked to higher rates of mineralization (Brunetto *et al.,* 2011). In this study none of the species had a C/N value greater than 25% (Table 1). The remaining phosphorus percent in the plant residues were 0.65 %, 2.72 % and 3.41 % for *C. micans, C. mucunoides* and *A. indica* respectively (Fig. 3 b). The loss of phosphorus shown by three legume species during the early stages of decomposition may be due to the release of soluble P-containing compounds (Isaac and Nair, 2005). Palm *et al.* (2001) recommended that high nitrogen (> 2.5%) and phosphorus (> 0.25%) during decomposition enhance mineralization, and in this study the phosphorus content in *C. micans* and *A. indica* was higher than *C. mucunoides* (Table 1) which could be the reason for the fast release of phosphorus in both the species. The overall inorganic phosphorus content and soluble phosphorus in the residues, as well as the effective activity of microbes in the organic fractions directly influence the release of phosphorus (Giocomini *et al.,* 2003).

This study provides valuable insights into the decomposition and nutrient release patterns of three leguminous weeds in Mizoram, with potential for use as green manure crops. *C. micans* decomposed fastest, followed by *C. mucunoides* and *A. indica. C. micans,* and *A. indica* released nutrients in the order of K > P > N, while *C. mucunoides* released them in the order of K > N > P. Overall, this study suggests using locally available leguminous plants as green manure due to their rapid decomposition rate.

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