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Decomposition and nitrogen mineralisation of two wild lupins (Leguminosae) species with potential as green manures

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ABSTRACT

The amount of biomass produced by various native species of genus *Lupinus* (L.) growing in Mexico ranges from 2.9-8.2 Mg/ha of dry matter, which can add up to 200 kg/ha of N to soil as green manure. However, information is scarce on the decomposition and mineralisation this biomass in the soil. The above-ground decomposition and N mineralisation of *Lupinus mexicanus* Cerv. ex Lag. and *Lupinus rotundiflorus* M.E. Jones species from Mexico using fine-mesh litter bags was evaluated. Litter bags containing 5 g of above-ground air-dried biomass at the vegetative and flowering stages were buried at a depth of 20 cm. Were dug up every 3 weeks over the course of 4 months, dried and re-weighed to determine the lost mass and total N by the Kjeldahl method. The largest decrease in residue mass occurred during the first 3 weeks of incubation. However, the lost mass was higher in younger green manure (75 days old) than in older plants (85 days old) after 18 weeks of exposure in the field. It was found that 60-75% of the total N in the plant material was released in the first 6 weeks. In *L. rotundiflorus* green manure, it was found that 79.14% of the initial N in the vegetative stage and 77.6% of N in the flowering stage was released 18 weeks after litter bag installation, whereas *L. mexicanus* were 74.6% and 74.7%, respectively. It was found that both decomposition and N mineralisation occurred quickly in the green manures evaluated.

Keywords: litter bags, legumes, biomass, soil, nutrient release

INTRODUCTION

The value of leguminous green manure crops in improving soil fertility has been recognised since very early times. Due to their ability to fix atmospheric nitrogen (N), both crop and native species of legumes have significantly greater above-ground dry-matter production and N content than non-legumes (Askegaard and Eriksen, 2007). Under favourable conditions, legumes can produce up to 8,000-10,000 kg/ha of dry matter (Bender and Tamm, 2014; Ingver et al., 2019), which can add up to 300 kg/ha of N into the soil (Lauringson et al., 2013). Some legumes are valuable as green manure crops due to their relatively low lignin content and C to N ratio, resulting in rapid decomposition and release of N (Brunetto et al., 2011). In this regard, legumes decomposing in soil represent an alternative or supplementary source of N that can reduce fertiliser N requirements for crops during the subsequent season (Poffenbarger et al., 2015). Amato et al. (1987) reported that, where leguminous residues and green manures are ploughed in, extensive net N mineralisation often occurs within 3-4 months of incorporation. Among the legumes with potential for use as green manure are the species of genus *Lupinus*. These species are widely grown in temperate regions of the world for grain, forage, and green manure, and they are more tolerant to acidic soil conditions then other legume crops (Zamora et al., 2017). Some species can accumulate 160-224 kg/ha yr N in biomass on average (Lauringson et al., 2013; Ingver et al., 2019; Zapata et al., 2019). Although the influence of different lupin species as green manure on soil N availability and vegetable crop yields has been reported in several studies (Takunov and Yagovenko, 2000; Cavigelli and Thien, 2003; Fowler et al., 2004), there is little information available on the decomposition and N release of lupins in field conditions when applied as a green manure (Alisson et al., 2017). In Mexico, lupin species are not known in cultivated form, but there are an estimated 100 native species (Zapata et al., 2019), and some of them have shown potential as forage and green manure. In Jalisco, Mexico, *Lupinus exaltatus*, *L. rotundiflorus* and *L. mexicanus* have been reported to be useful as N suppliers in various cropping systems due to high plant N content and dry matter production (Zapata et al., 2019). However, it is also necessary to know the rate of decomposition of plant biomass into the soil and subsequent release of N, which is fundamental when choosing a species that is adapted to local conditions.

The objective of this study was to evaluate the decomposition and N release of green manure herbage from *Lupinus mexicanus* Cerv. ex Lag. and *L. rotundiflorus* M.E. Jones at different growth stages using the buried mesh litter bag technique.

MATERIALS AND METHODS

Experimental site and soil

The field experiment was conducted in an agricultural plot located in Zapopan Jalisco (20°43′ N, 103°23′ W), Mexico during the summer of 2018 (1 July to 30 October). At this site, the mean annual temperature is 19.7 °C to 23.5 °C, and the average annual rainfall is 798 to 890 mm. The soil type is classified as *Regosol* according to the Classification System of the Food and Agriculture Organisation (FAO). Chemical analysis of the soil in the 0-20 cm layer showed the following characteristics: texture, sandy loam; pH (H_2O), 5.9; organic matter, 1.6%; available P, 30 mg/kg, K, Ca, and Mg and cation exchange capacity (CEC), 0.59, 4.5 and 0.91 cmol/kg, and 11.8 cmol/kg, respectively. The climatic conditions during the experimental period are shown in figure 1.

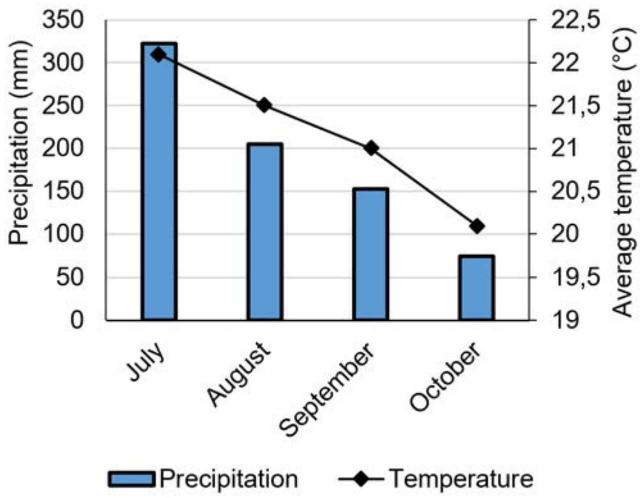

Figure 1. Monthly rainfall and average temperature during the field incubation

Plant material

Green manure of *Lupinus mexicanus* Cerv. ex Lag. and *L. rotundiflorus* M.E. Jones, defined here as above-ground plant parts, was produced and collected in a field experiment at the University of Guadalajara in Zapopan, Jalisco, Mexico as described by Zamora et al. (2017). The soil properties and climatic conditions at this site were described previously (Zapata et al., 2019). The plants were grown from November 2017 to April 2018 prior to the onset of the decomposition and N release experiment. The above-ground biomass of *L. mexicanus* and *L. rotundiflorus* in the vegetative and flowering stages was harvested 75 and 85 days after being sown, respectively.

All plant material was washed and chopped into pieces with a maximum length of 2 cm. Prior to incubation in the soil, the plant material was air dried in the greenhouse to a constant weight. After drying, ground samples were analysed for total C, N and biomass fractions (hemicellulose, cellulose and lignin), and C to N ratio at the Biotechnology Laboratory of the University de Guadalajara, Mexico (Table 1). The total C and N content of the green manure was determined by dry combustion using an elemental analyser (CHSN-O LECO TruSpec®, Leco Corporation). Lignin, cellulose, and hemicellulose contents were analysed by the acid detergent fibre method. These samples represent the starting values before decomposition.

Table 1. Initial chemical composition of different wild lupin species used in the decomposition experiment

	Initial concentration %					
Growth stage	N	C	Lignin	Cellulose	Hemicellulose	C:N
			Lupinus mexicanus			
Vegetative	2.49	38.6	18.89	35.26	14.25	15.5
Flowering	2.24	40.0	20.96	37.95	13.16	17.8
			Lupinus rotundiflorus			
Vegetative	2.92	39.5	18.62	35.09	17.38	13.53
Flowering	2.76	40.2	20.92	38.12	16.60	14.56

N = nitrogen, C = carbon, C:N = carbon:nitrogen ratio

Litter bag preparation and processing

Nylon mesh 5 × 10 cm litter bags (Ankom Scientific, USA) with 50-µm openings and filled them at a rate of 5.0 g per bag on a dry matter basis were used. The litter bags were weighed empty and again after adding the plant material. The bags were closed at the top by folding the top 3 cm of the bag over and stapling twice. An aluminium tag with the bag ID was stapled to each bag. Bags with plant material were stored at 5 °C until the field experiment.

Experimental design

For the litter bag study, a randomised complete block design with factorial arrangement and three replicates was used at both locations. The experimental factors at each location were two green manure species (*L. mexicanus* Cerv. ex Lag. and *L. rotundiflorus* M.E. Jones), two different growth stages (vegetative and flowering stages), and six sampling times (3, 6, 9, 12, 15 and 18 weeks). A total of 24 treatments and three repetitions were compared. Litter bags containing lupin green manure at different growth stages were randomly assigned and buried at a depth of 20 cm on 1 July 2018.

Samples and calculations

Three bags, which served as three replicates for each treatment, were dug up 3, 6, 9, 12, 15 and 18 weeks after study initiation to monitor the dry matter and N losses. The nylon bags collected at each sampling time were manually cleaned to remove soil particles. The plant materials remaining in the nylon bags were oven dried at 65 °C to constant weight to determine dry matter losses and perform a total N analysis using the Kjeldahl method (Bremner, 1996).

The percent weight loss for each period was calculated as $100 \times (M_o - M_t) / M_o$, where M_o is the initial plant material dry matter mass (g) in the nylon bag, M_t – the plant material dry matter mass (g) in the bag at time t, when the bags were removed from the field.

The amount of mineralised N (N_m; % of initial amount) over the sampling time (t) was calculated using the formula:

$$N_m = 100 \times (N_i - N_t) / N_i,$$

where N_i is the initial amount (mg) of nitrogen in the decomposing material, N_t – the amount (mg) of N in the decomposing material at time t.

Statistical analysis

To analyse the data, the statistical software Statgraphics Centurion, version XVII (Statgraphics Technologies Inc., USA) was used. Analysis of variance (ANOVA) was performed for each variable (% weight loss and % N mineralised) and Tukey's test used to establish significant differences among means. To indicate significance, $P<0.05$ was considered.

RESULTS AND DISCUSSION

According to the factorial ANOVA, lupin green manure species, growth stage, and incubation time had a significant effect on the mass remaining and nitrogen (N) released (Table 2).

Decomposition of lupin green manure

Decomposition (measured as a loss in weight of the green manure) over time varied significantly between phenological stages and between species of lupins tested. However, the differences in decomposition rate estimates were generally small. As expected, the mass weight of both *L. rotundiflorus* and *L. mexicanus*, either the vegetative (70 days old) or flowering stage (85 days old), decreased significantly with time (Figure 2).

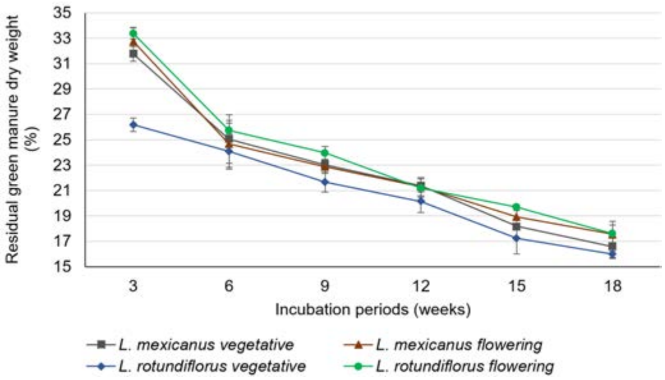

Figure 2. Percentage of remaining biomass (dry weight) of lupin green manures buried in the soil at different phenological stages

This observation is in agreement with findings from other studies with different legumes used as green manure (Fosu et al., 2007; Duarte et al., 2013; Pereira et al., 2016; Saria et al., 2018).

There was a slight tendency for greater loss of weight in green manure at the vegetative stage (70 days old) than in the flowering stage (85 days old), but it was more marked in the first weeks. The largest loss in green manure mass occurred after the first 3 weeks, with an average value of 73.8% in the vegetative stage and 66.6% in the flowering stage for *L. rotundiflorus*, whereas these values were 68.24% and 67.25%, respectively, for *L. mexicanus*. This decrease in green manure mass coincided with the first rains and favourable temperatures (Figure 1), whereas successive rainfall events did not correspond with a similar decrease in mass. The fast weight loss found in the first weeks is in agreement with Nyberg et al. (2002), who reported that the initial phase of decomposition is characterised by a rapid loss of hydrosoluble compounds, high microbial activity, and leaching/release of nutrients. Between weeks 6 and 12, the loss of mass by both species was more gradual, and these losses were minimal between 15 and 18 weeks, probably due to the low recorded rainfall and the accumulation of recalcitrant components, such as lignin and cellulose, which protect cell walls from attack by microorganisms (Thönnissen et al., 2000). In general, our results on the fast initial decomposition of

Table 2. Factorial ANOVA of the effects of the species used as green manure and growth stage on the biomass remaining and nitrogen (N) mineralised

	Remaining biomass g					N mineralised %				
Source	gl	SC	CM	F	*P*-value	gl	SC	CM	F	*P*-value
Species (S)	1	0.02	0.02	8.04	<0.0066*	1	132.5	132.5	98.3	<0.0001*
Growth stage (Gs)	1	0.11	0.12	52.31	<0.0001*	1	22.36	22.36	16.59	<0.0001*
Time (T)	5	3.82	0.76	378.3	<0.0001*	5	489.74	97.95	72.65	<0.0001*
S × Gs	1	0.06	0.06	31.23	<0.0001*	1	1.94	1.94	1.44	<0.2365 ns
S × T	5	0.03	0.01	3.38	<0.0107*	5	74.60	14.92	11.07	<0.0001*
Gs × T	5	0.06	0.01	6.41	<0.0001*	5	104.83	20.97	15.55	<0.0008*
S × Gs × T	5	0.04	0.01	3.63	<0.0073*	5	162.71	32.54	24.14	<0.0001*

gl = degrees of freedom, SC = sum of squares, CM = mean square, * = statistical significances P<0.05, ns = non-significant at a P<0.05

both lupin species are in accordance with the findings in other studies of legumes used as green manure. For example, Tönnissen et al. (2000) found that soybean and *Indigofera tinctoria* green manures decompose rapidly, losing 30 to 70% of their biomass within 5 weeks. On the other hand, Lee et al. (2002) found that hairy vetch (*Vicia villosa*) loses 72-81% of its weight after 1 month. In Brazil, Matos et al. (2011) conducted a litter bag study of legume decomposition (*Arachis pintoi*, *Calopogonium mucunoides*, *Stizilobium aterrimum*, and *Stylosanthes guianensis*) and found rapid decomposition (average 30-45% of weight lost after 1 month). However, a recent study in Brazil reported a 22% loss of weight for *Lupinus albus* over a period of approximately 3 weeks of incubation, which is lower than that recorded in this study and indicates a greater resistance to decomposition (Alisson et al., 2017).

On the other hand, early investigations have shown that the concentrations of lignin, N, C:N and polyphenols in the plant material are important characteristics governing the rate of decomposition (Müller et al., 1988). Taking into account that the green manures in our study had some differences in their chemical compositions, the greater decomposition of *L. mexicanus* compared to *L. rotundiflorus* can be explained by initial differences in the average N content and C to N ratio (Table 1). Traditionally, the C to N ratio has been used as the best index for predicting decomposition and N mineralisation in leguminous and non-leguminous green manures (Lee et al., 2002; Watthier et al., 2020).

Nitrogen (N) mineralisation

Significant differences in mineral N release were found between plant species and between phenological stages. The amount of N mineralised in field studies varies depending on many interacting factors, such as the green manure species, chemical composition of the plant material, soil and climatic conditions, technique, and time for incorporation into the soil. Previous studies with different green manures applied to the soil have shown more rapid N mineralisation when the overall plant N concentration is high (%N > 2% or C:N < 20) (Cordovil et al., 2005; Masunga et al., 2016). In our study, the largest net N release in both green manures occurred during the first 3 weeks of incubation (63-72%), but in subsequent weeks N mineralisation did not increase significantly (Figure 3).

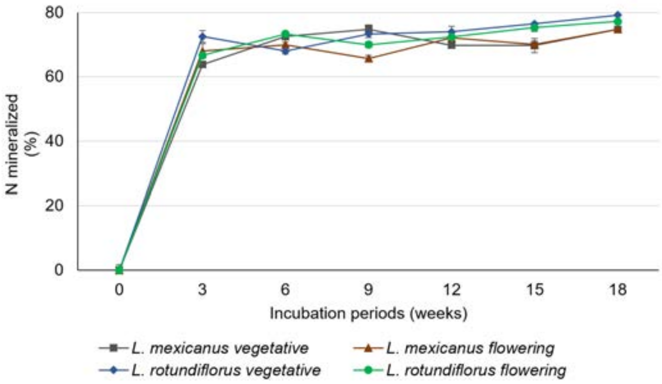

Figure 3. Percentage of mineralised nitrogen (N) in lupin green manures

The highest percentage of mineralised N was found in *L. rotundiflorus* with 72.5% in the vegetative stage and 66.64% in the flowering stage in the first weeks after placing the litter bags in the field, whereas the percentage of N mineralised in *L. mexicanus* was 67.9% and 63.8%, respectively. Other authors have also reported initial net N release in experiments with different green manures with low C to N ratio (Lee et al., 2002; Watthier et al., 2020). According to Bonanomi et al. (2021), when the C to N ratio is >30, microbial growth, organic matter decomposition, and the associated N mineralisation rate decrease because of N starvation. The amount of N released in *L. rotundiflorus* at the end of the experiment was equivalent to 23.3 g/kg N biomass in the vegetative stage and 21.13 g/kg N biomass in the flowering stage; in *L. mexicanus*, the values were 18.46 and 15.48 g/kg N biomass, respectively. Between 6 and 15 weeks, both *L. mexicanus* and *L. rotundiflorus* exhibited short-term immobilisation, followed by slow N mineralisation until the end of the experiment. In our study, the greater net N mineralisation from younger plants (vegetative stage) compared to older plants (flowering stage) could be explained by differences in the chemical composition. The small differences in N release between plants of different growth status (i.e., different age) could also be related to small differences in the N and lignin content (Table 1). As the plants mature, they generally tend to decline in quality due to an altered chemical composition involving lignification. Lignification of cell walls during plant development has been identified as the major factor limiting the decomposition and N mineralisation of green manure crops. A previous study reported that a higher lignin concentration in plants protects N from being mineralised (Müller et al., 1988). On the other hand, according to Haynes (1986), plant lignins reduce net N mineralisation because they are degraded to polyphenols, which are initially present in the plant and combine with nitrogenous compounds to form recalcitrant humic polymers. Our study confirms that, on the local scale, where climatic conditions are relatively uniform, the interaction of the chemical composition of the plant tissue with soil microorganisms is the most important factor determining the rate of organic matter decomposition and mineral N release (Bradford et al., 2017; Bonanomi et al., 2021).

CONCLUSION

Above-ground decomposition and N mineralisation were fast in both green manures. The green manures released N faster in the vegetative stage (70 days old) than in the flowering stage (85 days old) regardless of species. In addition, the differences in N release between plants of different growth status could be related to small differences in biomass lignin content. *Lupinus rotundiflorus* and *Lupinus mexicanus* can be successfully used as a green manures to increasing fertility of poor sandy soils from Jalisco México.

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