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# EFFECT OF MOUNTAIN MICROORGANISMS ON DECOMPOSITION AND NUTRIENT RELEASE IN TWO GREEN MANURES

Isidro Zapata Hernández[1], Carlos Ernesto Aguilar Jiménez[1], José Galdámez Galdámez[1], Juan Carlos López Hernández[1], Juan Francisco Zamora Natera[2]

[1] Autonomous University of Chiapas, Faculty of Agronomic, Sciences Campus V. Km. 87 Road Tuxtla Gutiérrez-Villaflores, Chiapas. C.P. 30470. Villaflores, Chiapas, Mexico

[2] Department of Botany and Zoology, University of Guadalajara, 45100, Nextipac, Zapopan, Jalisco, Mexico

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## Abstract

The aim of this research was to evaluate the decomposition and release of nitrogen, phosphorus, and potassium in the biomass of two green manures: *Canavalia ensiformis* (L.) and *Mucuna deeringiana* (Bort) Merr. After incubation in the soil, they were treated with mountain microorganisms (MM). An experiment was proven in the experimental agricultural field of the Faculty of Agronomic Sciences of the Autonomous University of Chiapas, Mexico, in Villaflores, Chiapas. The decomposition bag technique was used, and the following treatments were evaluated: *C. ensiformis*+MM, *C. ensiformis*–MM, *M. deeringiana*+MM, and *M. deeringiana*–MM. A randomized block design with a factorial arrangement was used; each bag with 10 g of dry matter inside was buried 15 cm deep in the soil. Three bags per treatment (six samplings) were removed every two weeks from the soil. The material was recovered, and the remaining dry biomass was quantified by difference. N was determined by the Kjeldahl method, P by colorimetry and K by atomic absorption spectrophotometry. Significant differences ($p < 0.05$) in the evaluated variables showed a gradual decomposition and nutrient release trend. *C. ensiformis*+MM showed higher decomposition with 97.68% and 93.02% in *C. ensiformis*–MM, while *M. deeringiana*+MM, and *M. deeringiana*–MM obtained 83.94% and 82.82%. The release of N, P, and K, irrespective of treatments, showed ranges of 44.77–253.97, 8.13–44.49 and 4.75–27.35 mg/10 g green manure, respectively. The application of MM in green manures accelerated their decomposition, and release of nutrients to the soil.

Keywords: NPK, *Canavalia ensiformis*, *Mucuna deeringiana*, soil fertility, organic matter, plant residues

## INTRODUCTION

Crop production has been affected by decreased soil fertility. This presents different erosion degrees, low organic matter content and low nutrient retention capacity (Adekiya, 2019). Therefore, agrochemicals have increased to support production and improve soil fertility. However, this has generated problems in its deterioration (Agbede *et al.* 2018). Thus, the negative impact of agrochemicals on the soil is one of the main environmental problems worldwide (Tejada *et al.,* 2008). The recovery of agricultural soil fertility in cropland areas is one of the great challenges for developing countries where strategies are sought to improve fertility and sustainability (Maitra *et al.,* 2018). One of the alternatives used for their potential as a source of organic matter and nutrients is green manures, which rely on their chemical composition and decomposition to improve soil conditions (Agbede *et al.,* 2018). Decomposition is a process by microorganisms; the decomposition and nutrient release rate depend on them. Currently, in Latin America, biofertilizers are produced locally for organic and ecological production known as biol or biols, where the source of inoculation is mountain microorganisms (MM) (Ney *et al.,* 2020), which are made from decomposing substrates collected from different diversified ecosystems and where no agrochemicals have been used (Rodríguez-Calampa and Tafur-Torres, 2014), these biofertilizers present fundamental functions in soil biological properties, availability of essential nutrients, residue decomposition, as well as pathogen suppressors (Martinez *et al.,* 2014). The main components of mountain microorganisms include nitrogen-fixing microorganisms, phosphorus solubilizers, lactobacilli and yeasts. Despite having a high microbiological load, it has low levels of nutrients; therefore, applying them to the soil together with green manures or other ecological practices favors the edaphic system (Castro *et al.,* 2015). Ney *et al.* (2018) mention that, despite being quite widely used, few studies related to the benefits and changes in soil ecology when applied. For this reason, the goal of the present study was to evaluate the effect of MM on the decomposition and release of N, P, and K from *Canavalia ensiformis* (L.) and *Mucuna deeringiana* (Bort) Merr. as green manures in Chiapas, Mexico.

## MATERIALS AND METHODS

### Location of the Experimental Site

The experiment was conducted in April–July 2021 at the Centro Universitario de Transferencia de Tecnología (CUTT San Ramón) of the Faculty of Agronomic Sciences of the Autonomous University of Chiapas, found in the municipality of Villaflores, Chiapas, Mexico (16°15' N and 93°14' W). It presents an altitude of 610 m.a.s.l., with a mean annual temperature of 22 ºC and accumulated yearly precipitation of 1 200 mm (Aguilar *et al.,* 2019). The climatic conditions during the experiment are shown in Tab. I. Soil analyses at 0–20 cm depth showed the following characteristics: pH, 4.02; organic matter, 1.41%; total N, 0.07%; P, 11 mg/kg; K, 0.3; Ca, 2.1; Mg, 0.5; Na, 0.1; cation exchange capacity (CEC), 8.3; texture, sand, clay, and silt, 55%, 26% and 19%, respectively.

I: *Average temperature and precipitation during the incubation experiment*

| Month | Average temperature | Precipitation (mm) |
|---|---|---|
| April | 28.33 | 0.00 |
| May | 27.83 | 198.94 |
| June | 26.93 | 270.93 |
| July | 26.90 | 130.02 |

### Plant Material

The green manures used were *C. ensiformis* and *M. deeringiana*, collected in April 2021 from an experimental green manure plot of the CUTT San Ramon of the Faculty of Agronomical Sciences, Campus V. The plants were in the phenological stage of fruiting (harvest residue). The samples were washed and cut into fractions smaller than 2 cm; the plant material was then dried at room temperature to constant weight, after which part of the sample was crushed for first analysis of N, P, K and C/N ratio (Tab. II).

II: *Initial N, P, and K from Canavalia ensiformis and Mucuna deeringiana as green manures*

| Green manure | Initial concentration mg/10 g plant material | | | |
|---|---|---|---|---|
| | C/N | N | P | K |
| *C. ensiformis* | 14 | 260 | 33 | 28 |
| *M. deeringiana* | 15 | 292 | 53 | 31 |

### Activated Mountain Microorganisms

The MM were collected from undisturbed forests around Villaflores Chiapas, after harvesting, the MM were mixed with equal parts of wheat bran and molasses dissolved in water (5 kg each), and a moisture content of 70 % was applied. The mixture was placed and compacted in a 20 L container to generate fermentation and reproduction of the MM. It was then hermetically sealed for 30 days. Then in a 200 L container containing 180 L of water and 20 kg of molasses, a blanket bag with the reproducing MM was placed. The 200 L container was hermetically sealed for a further 30 days. At the end of the incubation period, the MM were in optimal conditions for use (Suchini, 2012; Torres-Pérez *et al.*, 2022). The microbiological analysis (Tab. III) shows the microbial biodiversity in the MM, provided by the Academic Corps in Sustainable Agriculture (UNACH-CA-114) of the Faculty of Agronomic Sciences Campus V of the Autonomous University of Chiapas.

III: *Microbiological Analysis of Mountain Microorganisms*

| Culture medium | Dilution | CFU/mL | Microorganisms |
|---|---|---|---|
| LB | $10^4$ | $> 10^4$ | Bacteria |
| | $10^6$ | $7 \times 10^8$ | |
| BK | $10^4$ | $> 10^4$ | g-Proteobacteria, *Pseudomonas* spp. |
| | $10^6$ | $1.15 \times 10^9$ | |
| MN | $10^4$ | $> 10^4$ | *Bacillus* spp. |
| | $10^6$ | $1.4 \times 10^8$ | |

LB: Luria-Bertani culture medium; BK: King's B culture medium; MN: nutrient medium; CFU/mL: colony forming units

### Experimental Setup and Design

The following treatments were evaluated: *C. ensiformis*+MM, *C. ensiformis*–MM, *M. deeringiana*+MM, and *M. deeringiana*–MM incubated in soil–the method known as litter bags was used. Nylon bags with a porosity of 0.5 mm, 10 × 10 cm, were used. Each bag held 10 g of green manure on a dry basis. Once the bags were closed, the MM was applied to the soil by spraying the complementary treatments at a concentration of 50 % (5 L MM + 5 L $H_2O$). The bags were then buried 15 cm deep in the soil. A randomized complete block experimental design with factorial arrangement was used. The factors were:

- Factor A = species used as green manure, with two levels (*C. ensiformis* and *M. deeringiana*),
- Factor B = MM with two groups (with MM+ and without MM–), and
- Factor C = incubation time in the soil with six levels (2, 4, 6, 8, 10 and 12 weeks after incubation) generating a total of 24 treatments with three replications (three bags per replication).

### Sample Collection and Calculations

Samples were collected in triplicate for each treatment for 2, 4, 6, 8, 10, and 12 weeks after the start of the experiment. The nylon bags were dug up and then taken to the laboratory to clean the soil particles manually; the remaining plant material was dried in an oven at 65 °C for 48 h, and then its dry weight was obtained. Nutrients N, P, and K were calculated by the Kjeldahl method (Bremner, 1996), by colorimetry (Chapman *et al.,* 1984), and atomic absorption spectrophotometry (Fishman and Downs, 1966), respectively. Decomposition (%) and nutrient release (mg) were calculated as a function of weight loss of plant material incorporated into the soil over time using the formulas proposed by Zamora-Natera *et al.* (2022) respectively:

$$D = 100 \times (M_0 - M_t)/M_0 , \quad (1)$$

$D$ .......the decomposition of the plant material,
$M_0$.....the first dry plant material (g) in each sample,
$M_t$......the plant material (g) obtained at each sampling time.

$$NPK = 100 \times NPK_i - NPK_t)/NPK_i , \quad (2)$$

$NPK$.....the nutrient release (NPK),
$NPK_i$....the number of first nutrients (mg) in the decomposing plant material, and
$NPK_t$....the number of nutrients in the decomposing plant material obtained at each sampling time.

### Statistical Analysis

Data on the percentage of decomposition and release of N, P, and K were subjected to multifactorial ANOVA. To name differences between means, Tukey's test was performed ($p \leq 0.05$). The statistical program Statgraphics Centurion XVII (Statgraphics Technologies Inc., USA) was used.

## RESULTS AND DISCUSSION

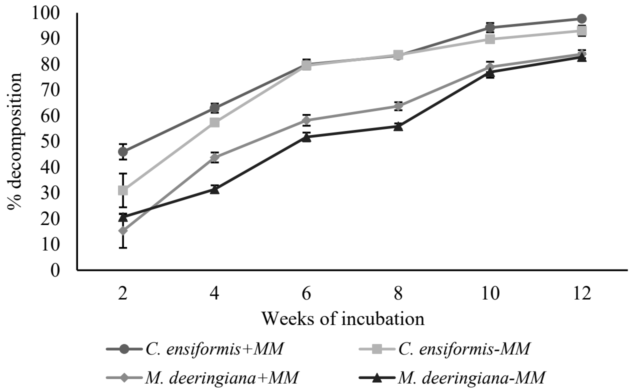
The effect of the MM applied to the green manures showed significant differences ($p < 0.05$) in the variables evaluated (% decomposition, N, P, and K released) (Tab. IV).

### Percentage of Decomposition

Decomposition in weight loss presented significant differences between treatments ($P < 0.05$). In general, a trend of gradually increasing decomposition was seen, where *C. ensiformis*+MM and *C. ensiformis*–MM presented the highest decomposition at the end of the experiment (97.68 %, 93.02 %, respectively) compared to *M. deeringiana*+MM (83.94 %) and *M. deeringiana*–MM (82.82 %) (Fig. 1). This relates to what Ibrahim *et al.* (2010) reported, who pointed out that applying efficient microorganisms with organic residues favors their rapid decomposition. Therefore, effectively managing green manures with MM under favorable conditions presents a helpful constructive interaction for their decomposition (Castro *et al.,* 2015). In the first six weeks, decomposition percentages ranged from 51.76 % in *M. deeringiana*–MM to 79.53 % in *C. ensiformis*+MM; these results are similar to those reported in decomposition studies with different legume species used as green manures (Matos *et al.,* 2011; Zamora and Zapata, 2021). The decomposition of green manures is related to their chemical and biochemical composition (C/N ratio, total N content,

IV: *Analysis of variance percentage of decomposition, N, P, and K released*

| Source of variation | gl | *Value P* | | | |
|---|---|---|---|---|---|
| | | Decomposition | N | P | K |
| Weeks of incubation | 5 | < 0.0001 | < 0.0001 | < 0.0001 | < 0.0001 |
| Treatments | 3 | < 0.0001 | < 0.0001 | < 0.0001 | < 0.0001 |
| Weeks of incubation *treatments | 15 | < 0.0001 | < 0.0001 | < 0.0001 | < 0.0001 |
| CV % | | 5.21 | 5.13 | 4.97 | 5.13 |
| $R^2$ | | 0.99 | 0.99 | 0.99 | 0.99 |
| $R^2$ Adjusted | | 0.98 | 0.98 | 0.98 | 0.98 |




1: *Decomposition percentage of two green manures with added MM*

lignin content, among others) (Fan *et al.,* 2017; Adekiya *et al.,* 2019), which eases the degradation of sugars and more labile components (Cotrufo *et al.,* 2013). Monsalve *et al.* (2017) mention other factors related to soil characteristics (texture, moisture, pH, microbial activity, organic matter, salinity, and nutrient availability) involved in decomposition. During weeks six and eight of incubation, a slow decomposition trend was seen concerning the other incubation weeks. This can be attributed to decomposing the most resistant materials with molecules that resist degradation, cellulose, and lignin (Brunetto *et al.,* 2011; Cotrufo *et al.,* 2013).

### Release of N, P, and K

These macronutrients are essential for best plant development (De Castro *et al.,* 2015). N is part of chlorophyll and amino acids and generates plant growth, while phosphorus is found in DNA and RNA and is present in root and seed development. At the same time, K plays a key role in activating many plant enzymes (Potdar *et al.,* 2021). Therefore, N, P, and K play significant roles in plant development with a requirement in copious quantities (Basu, 2011); however, K as a macronutrient in agriculture has yet to have the importance given to P, and N (Guaya *et al.,* 2018) During the decomposition process, the release of nutrients to the soil occurs, where the recycling of N, P, and K is obtained (Carvalho *et al.,* 2015; Agbede *et al.,* 2018). Legume green manures supply considerable amounts of N derived from biological nitrogen fixation and improve the availability of P and K in the soil (Requejo and Eichler-Löbermann, 2014).

The highest N release occurred at 12 weeks of incubation, where the *C. ensiformis*+MM and *M. deeringiana*+MM treatments showed the maximum values of 253.97 and 245.12 mg/10 g of green manure, respectively, compared to the treatments not treated with MM (241.85 mg/10 g). The trend of increasing N released was gradual throughout the incubation (Tab. V). This pattern was seen in decomposition studies with different legume species with green manure potential (Dabin *et al.,* 2016; Zamora and Zapata, 2021).

The P released by the green manures during the incubation period was gradual until the end of the experiment. Among the treatments of *M. deeringiana* in incubation weeks four, six and eight, significant differences were showing better values for the MM treatment. While in the green manure of *C. ensiformis*, they were similar in all weeks of incubation (Tab. VI). After P is released and for it to be available, solubilization and mineralization of inorganic and organic P by microorganisms capable of carrying out these processes are necessary (Castro and González, 2021). The similar results at the end of the experiment are mainly due to P immobilisation, as microorganisms have the metabolic capacity to accumulate polyphosphates intracellularly (Fukushima *et al.*, 2010) and also to P acquisition by several bacteria of the Firmicutes and Gamma proteobacteria group, as they have

V: *N released (mg/10 g) during incubation of green manures in soil*

| Treatment | Weeks of incubation | | | | | |
|---|---|---|---|---|---|---|
| | 2 | 4 | 6 | 8 | 10 | 12 |
| MD–MM | 60.35b | 91.88d | 151.16c | 163.09c | 224.80b | 241.85b |
| MD+MM | 44.77b | 127.90c | 170.04b | 185.90a | 230.58b | 245.12ab |
| CE–MM | 80.60ab | 149.24b | 206.79a | 217.45a | 233.25ab | 241.85b |
| CE+MM | 119.60a | 163.71a | 207.82a | 216.69a | 245.02a | 253.97a |

MD-MM: *M. deeringiana*–MM; MD+MM: *M. deeringiana*+MM; CE-MM: *C. ensiformis*–MM; CE+MM: *C. ensiformis*+MM. A column with different letters stands for significant differences (p = 0.05).

VI: *P released (mg/10 g) during incubation of green manures in soil*

| Treatment | Weeks of incubation | | | | | |
|---|---|---|---|---|---|---|
| | 2 | 4 | 6 | 8 | 10 | 12 |
| MD–MM | 10.95a | 16.67c | 27.44b | 29.60b | 40.80a | 43.90a |
| MD+MM | 8.13a | 23.22a | 30.86a | 33.74a | 41.85a | 44.49a |
| CE–MM | 10.23a | 18.94b | 26.25b | 27.60c | 29.60b | 30.70b |
| CE+MM | 15.18a | 20.78b | 26.38b | 27.50c | 31.10b | 32.23b |

MD-MM: *M. deeringiana*–MM; MD+MM: *M. deeringiana*+MM; CE-MM: *C. ensiformis*–MM; CE+MM: *C. ensiformis*+MM. A column with different letters stands for significant differences (p = 0.05)

VII: *K released (mg/10 g) during incubation of green manures in soil*

| Treatment | Weeks of incubation | | | | | |
|---|---|---|---|---|---|---|
| | 2 | 4 | 6 | 8 | 10 | 12 |
| MD–MM | 6.41b | 9.76d | 16.05c | 17.31c | 23.87b | 25.68b |
| MD+MM | 4.75b | 13.58c | 18.05b | 19.73b | 24.48b | 26.02b |
| CE–MM | 8.68ab | 16.07b | 22.07a | 23.42a | 25.12ab | 26.05b |
| CE+MM | 12.88a | 17.63a | 22.38a | 23.34a | 26.38a | 27.35a |

MD-MM: *M. deeringiana*–MM; MD+MM: *M. deeringiana*+MM; CE-MM: *C. ensiformis*–MM; CE+MM: *C. ensiformis*+MM. A column with different letters is significant differences (p = 0.05)

the ability to synthesise organic acids which they subsequently use to transfer phosphate ions from the exchange sites for availability (Ogut *et al*., 2010). So the use of MM can increase the amount of P available in the soil solution (Castro *et al.,* 2015).

K release from green manures showed significant differences in all incubation weeks. The highest treatment represented was *C. ensiformis*+MM (27.35 mg/10 g). In contrast, the *M. deeringiana*–MM treatment was the one that presented lower values than the other treatments, with 25.68 mg/10 g at the end of the experiment (Tab. VII). Leite *et al.* (2010) mention that K release in legumes is rapid because it is not part of structural components and is found in the vacuole of plant cells in ionic form. Moreover, its release in soil depends on the K diffusion factor, which is influenced by moisture and precipitation and not necessarily by microbial processes (Crusciol *et al.,* 2008; Leite *et al.,* 2010).

The decomposition and release of nutrients present an increase when the activity and quantity of soil microorganisms and enzymes are increased (Yang *et al.,* 2014); therefore, the higher decomposition and release of N, P, and K in MM treatments can be attributed to the higher microbial activity, as it performs the mineralization of organic components faster (Shaheen *et al.,* 2017). In addition, Willey *et al.* (2011) also mentioned that these types of microorganisms function in a most excellent soil temperature range of 20 to 45 °C, with a minimum temperature of 15 °C where these microorganisms thrive. In this study, the soil temperature was within the mentioned range. The results obtained for P and K release are similar to those reported for 240 days of decomposition by Mendonça *et al.* (2018), while N results obtained higher values in this study.

## CONCLUSION

Despite presenting the same trend of decomposition and release of N, P, and K in all treatments, the application of MM on *C. ensiformis* and *M. deeringiana* increased the decay and release of N, P, and K in the soil. Regardless of the application of MM, *C. ensiformis* and *M. deeringiana* can be used as green manures due to the increase of nutrients they leave available in the soil each time. MM together with green manures benefited soil nutrition. Therefore, their use can help degraded soils to improve their quality in a sustainable way, favouring organic agriculture and agroecology.

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Contact information

Isidro Zapata Hernández: chilo0602@hotmail.com
Carlos Ernesto Aguilar Jiménez: ejimenez@unach.mx (corresponding author)
José Galdámez Galdámez: jose.galdamez@unach.mx
Juan Carlos López Hernández: lopezhjuancarlos@hotmail.com
Juan Francisco Zamora Natera: jfzamora@cucba.udg.mx